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by

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AND

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WITH A FOREWORD BY

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 6 **April, 1960** *Part 1*

Tests for Liver Function in Domestic Animals

BY

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INTRODUCTION

The purpose of this review is to summarize work done on liver function in the veterinary field, with special reference to tests for liver function and their diagnostic use in small-animal medicine.

The study of liver function is made difficult by the complexity of the liver and its multiplicity of functions. Also it has amazing powers of regeneration: to quote Sir Heneage Ogilvie in a recent book "No Miracles Among Friends" ... "The liver alone among the tissues of the body has the powers of survival and repair and regeneration of a primitive animal". In liver disease, according to the type and degree of damage, various functions or combination of functions will be affected. Therefore a range of liver function tests and a careful clinical examination may be necessary before a diagnosis can be made.

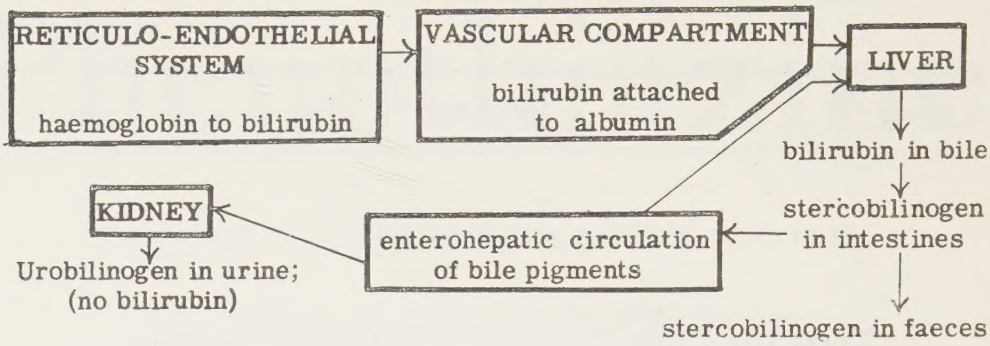
It is proposed to divide the functions of the liver into three groups; its secretory and excretory activities, its metabolic activities and its methods of detoxication.

PART I. SECRETORY AND EXCRETORY FUNCTIONS OF THE LIVER AND THE ASSOCIATED GROUP OF LIVER FUNCTION TESTS

Secretion of bile is probably one of the most important functions of the liver. Bilirubin is excreted in the bile and is broken down by bacterial activity in the intestines to form stercobilinogen, some of which

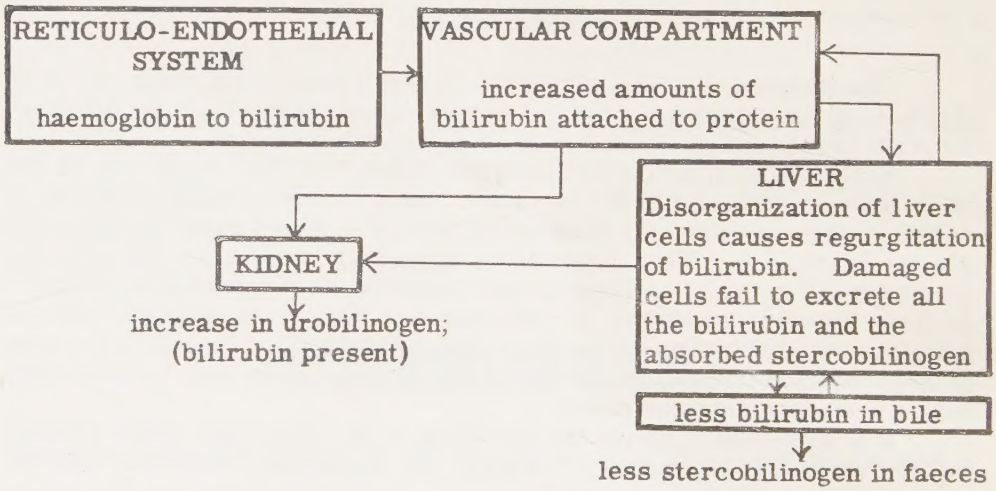
is reabsorbed by the colon and passes to the hepatic portal system, thus giving rise to the enterohepatic circulation of bile pigments. In the normal animal the small quantity of stercobilinogen which is not re-excreted by the liver is excreted by the kidney as urobilinogen.

This circulation of bile pigments in man can be represented by the following diagram (adapted from Sherlock, 1955).



In liver damage giving rise to hepatocellular jaundice, alterations will occur in the quantities of bile pigment found in blood, serum, urine and faeces.

These changes can be represented diagrammatically as follows (adapted from Sherlock, 1955).



1. Tests for bile pigments.

van den Bergh's Test. For many years some confusion has existed about the reaction of certain sera to the diazo test for bilirubin described by Hijmans van den Bergh. It has been recognized that when van den Bergh's reagent is added to serum from cases of obstructive or post-hepatic jaundice a prompt colour change occurs without the addition of alcohol. This has been called the "Direct Reaction". On the

other hand when the same reagent is added to sera from cases of haemolytic or pre-hepatic jaundice, a reddish-blue colour is formed only after the addition of alcohol, giving rise to the "Indirect Reaction". In cases of hepatocellular jaundice a biphasic reaction is observed, a reddish colour developing rapidly, but intensifying and becoming more blue on standing. Much work has been done to find the explanation for these different reactions, and this work has been very adequately described by Gray (1957). Briefly, the view now held is that the pigment present in "indirect reacting" serum is bilirubin itself, an insoluble compound. "Direct reacting" serum on the other hand, has been shown to contain two pigments, the monoglucuronide and diglucuronide of bilirubin (Billing and Lathe, 1956). These glucuronides are soluble compounds, and can be found in the urine in cases of hepatocellular and post-hepatic jaundice. Conjugation of bilirubin to produce these compounds probably takes place in the liver. Klatskin and Bungards (1956) have shown that bilirubin transported in the serum is mostly bound to serum albumin and that it does not form a globulin complex as had been suggested by Fiessinger et al. (1941, 1942).

Concerning the veterinary applications of van den Bergh's technique, Brouwers (1940) used White's (1932) modification which uses a solution of cobalt sulphate as a standard of comparison.

During tests on the sera of 100 normal dogs, 50 horses, 25 pigs, and 10 cattle Brouwers concluded that the bilirubin content of serum varied greatly from one species to another; while it remained low and constant in the dog, it was raised and variable from one horse to another. In cattle and pigs it remained low and stable. Brouwers tried to estimate the normal quantity of indirect bilirubin in sera in horses of similar age and breed, given a similar diet. He took precautions to use an identical technique in all tests, but in spite of this he observed great variance in the results. He estimated the average to be 15 mg. per 100 ml. in horses of five years or less with a range of 10–26 mg., and an average of 34 mg. per 100 ml. in aged horses with a range of 18–53 mg.

In horses with known hepatic disease Brouwers tried to find some correlation between bilirubin content of serum and the extent of the lesions. In spite of the presence of very considerable hepatic lesions which were confirmed at autopsy, including fatty degeneration, cirrhosis, and melanosis, it was not possible to establish any correlation. The only consistent variation from the normal was found in two cases of generalized echinococcosis where values of from 84–100 mg. bilirubin per 100 ml. were obtained.

Similar work by other authors supports Brouwers' view that the highest serum bilirubin levels are found in horses.

Various workers have used van den Bergh's reaction on the sera of cattle, sheep and pigs. These animals all show lower values than the horse, and exhibit more uniformity. Muzzo (1949) stated that the total serum bilirubin level averaged 0.14 mg.% in cattle, 0.1 mg.% in sheep and 0.09 mg.% in pigs.

Berger (1956) stated that bilirubin could not always be demonstrated in the serum of cattle, sheep, pigs and dogs, but he noted that withholding food caused a rise in the levels. Zink (1932) had also found that higher

values could be obtained in cattle and pigs after a period of so-called "starvation rations", for instance in cattle increases were observed from 0.25–0.47 mg.% after four days on a near-starvation diet. Pedini (1954) claimed good results by using the Jendrassik & Grof (1938) modification of van den Bergh's test on 52 cattle.

All workers have reported low or even negative values for healthy dogs. Brouwers quoted five positive tests for 100 sera examined, but pointed out that with the technique he used a part of the bilirubin became adsorbed to precipitated protein. Zink claimed a positive reaction in 30 out of 100 sera, with values up to 0.1 mg.%, and Berger also obtained readings of total bilirubin up to 0.1 mg.%.

Karsai (1954) also used the Jendrassik & Grof modification and found the total serum bilirubin of the dog to lie between 0.28–0.35 mg.%, the amount of direct bilirubin varying between 0.06–0.12 mg.%. Values obtained by the use of this method are usually higher than those obtained with the simpler colorimetric methods.

Both Brouwers and Karsai have examined serum bilirubin in dogs with liver disease. Brouwers has given details of five interesting clinical cases of dogs with serum bilirubin ranging from 7–25 mg. per 100 ml. It was concluded that the test was positive in most cases of jaundice in the dog and became more marked as cholaemia increased. Brouwers commented, however, that survival of jaundiced dogs bore no apparent relationship to the bilirubin content of serum, which depended more on the integrity of the renal parenchyma. Some dogs which survived had a higher bilirubin content than those which succumbed.

Karsai investigated 22 clinic cases. The highest total bilirubin content was 2.98 mg.%, obtained from a dog which at autopsy was found to have hepatitis, possibly leptospiral in origin. Serum from this dog had a direct bilirubin content of 1.85 mg.%.

The next highest total bilirubin content was 2.12 mg.% from a dog with enteritis, showing considerable liver enlargement. This dog recovered, so the liver could not be examined post mortem. The direct bilirubin content of serum in this case was 1.52 mg.%. Other values ranged from 0.29 mg.% in a case of renal cirrhosis to 0.89 mg.% in liver cirrhosis.

Karsai also studied four dogs experimentally poisoned with carbon tetrachloride (CCl_4) and phosphorus. He observed that the rise in the quantity of total bilirubin was always proportional to the severity of changes in the liver. He advised that in the diagnosis of latent cases of icterus the determination of total serum bilirubin was indispensable.

Hoerlein and Greene (1950) reported that two dogs with leptospiral jaundice gave total serum bilirubin levels of 4 mg.%, Ott (1956) stated that typical direct, indirect and biphasic reactions were obtained with sera from cases of obstructive, haemolytic and hepatocellular jaundice.

Gornall & Bardawill (1952) used a modification of van den Bergh's test described by Malloy & Evelyn (1937) and found that when liver damage was sufficient to raise bromsulphalein (BSP) retention to 30% and higher in dogs poisoned with CCl_4, the bilirubin content of serum increased to 0.8 mg.%. Instances were recorded, one with BSP retention at 54% and the other at 80%, in which the serum bilirubin increased to 2.3 and

3.7 mg.% respectively. When serum bilirubin exceeded 2 mg.% prognosis was poor. They also noted that the urine contained moderate amounts of bilirubin when the serum content was around 0.5 mg.% and it was concluded that the renal threshold of the dog was probably lower than in man.

Cornelius (1958) stated that by measuring both the total bile pigments with addition of alcohol and the so-called direct reacting glucuronide it was possible, by calculating the difference in these readings, to indicate the level of indirectly reacting pre-hepatic serum bilirubin, a procedure which was of assistance in the differential diagnosis of jaundice. A preponderance of indirect-reacting pigment indicated haemolytic jaundice. If over 50% of the pigments were direct-reacting the cause of the jaundice was usually extra-hepatic bile duct closure or intra-hepatic occlusion which was hepatogenous in origin.

Svirbely, Monaco & Alford (1946) concluded that the van den Bergh's reaction was of limited value in canine medicine, for they obtained positive results in dogs experimentally poisoned with xylidine only when the animals were near death.

To conclude this section it is interesting to note another variation of the van den Bergh's test, that of Gibson & Goodrich (1934), which was used by Smith et al. (1940) on cats and rabbits with experimental selenium poisoning. This eliminated the use of standards and uncertainties of colour matching. In this instance measurable amounts of bilirubin were not found, probably because bilirubinaemia was not a feature of liver damage caused by selenium poisoning.

Bilirubin in urine. Bilirubin is generally present in the urine in cases of hepatocellular or obstructive jaundice, and its presence can be demonstrated in several ways.

Heidrich (1954) used the methylene blue test to demonstrate bilirubin in urine of cattle. He used Franke's (1931) modification of the test and found that although pH of the urine did not affect the results, a positive result was always given by urine containing haemoglobin. Of 78 cattle with traumatic reticulitis 20 gave a positive methylene blue test, as did two out of three cases of acetonaemia. Urine from 60 of 100 cattle with proven liver disease gave positive results but the remaining 40 were negative. Heidrich concluded that the test should only be used if coupled with a careful clinical examination.

Lewis (1956) also used the methylene blue test. He claimed that its sensitivity was high and that positive results could be obtained in urine containing trace amounts of bilirubin not detectable by Gmelin's test. The natural pigment of urine, urochrome, did not produce a positive reaction possibly because there was a very distinct difference in the wavelengths of the different colours produced.

Krispien (1954) compared the methylene blue test with tests for bile pigment described by Ingham (1951) and Harrison (1930). The latter are more complicated than the methylene blue test because they involve concentration of pigments on a gel mass followed by detection of these pigments by special reagents. Results of the three tests on cattle could be divided into three groups. The first group gave positive results to the

tests of Ingham and Harrison but negative to methylene blue; the second group were positive to all three tests, and the last group were negative to the tests of Ingham and Harrison tests but positive to methylene blue. The first group of cattle appeared to be fairly healthy, the second group showed definite malaise, while the third showed signs of acute illness. Ingham's test appeared to be very sensitive but the technique was somewhat lengthy, particularly preparation of the gel. Harrison's test was quicker and it was easy to interpret. Both tests seemed specially good for diagnosis of early liver disease.

El-Gindy (1956) stated that the methylene blue test was simpler and more accurate than the tests of Ingham and Harrison. While methylene blue indicated positive results with bilirubin concentrations of 3 mg.%, 8 mg.% was necessary to give positive results to the other two tests.

Balloni (1934) used the barium chloride test described by Laemmer (1932) and modified by Severi (1934) to demonstrate bile salts in urine of domestic animals. Using urine of healthy dogs a white precipitate soon formed, and the top layer of urine changed from rose pink to yellow. In jaundiced dogs the precipitate was blue-grey and only became white after standing for 5–7 hours; urine colour ranged from greyish-pink to yellow after 5–6 hours.

Kapralov (1953) also described a test using barium chloride, but gave no data on its reliability.

Cornelius stated that as free bilirubin did not occur in urine the presence of bile pigment must be due to regurgitation of conjugated bilirubin in the liver following normal cellular conjugation. Since the renal threshold was low for this bile pigment it was an extremely sensitive test for early hepatocellular changes, as in leptospirosis, as well as bile duct obstruction. But it was difficult to interpret its presence in dog urine since bilirubin can be present in a low concentration in any febrile state.

Payne (1955) used a modification of the methylene blue test described by Gellis & Stokes (1945).

Hoerlein et al. used the methylene blue test on various clinical cases, and it is interesting to note that cases which gave high values for BSP retention and icteric index tests gave correspondingly high values with methylene blue.

A new test, using a reagent in tablet form marketed under the name "Ictotest", is based on the ability of bilirubin to couple with the diazo compound para-nitrobenzenediazonium-para-toluenesulphonate. Its use in human medicine was described by Sobotka et al. and Giordano & Winstead in the U.S.A. in 1953 and by Tallack & Sherlock in England in 1954. Although its use on dog urine is advocated by Ott, Payne & Cornelius there does not yet appear to be sufficient accurate information on its value.

Urobilinogen in urine. Wallace & Diamond (1925) described the identification of urobilinogen in urine and gave the results obtained with urine from normal dogs and from dogs with liver damage caused by CCl_4. Dogs poisoned with CCl_4 excreted an increased quantity of urinary urobilinogen proportional to the degree of liver damage. When

CCl_4 was administered before chloroform the effect was intensified. In two dogs urobilinogen tests were compared with phenoltetrachlorphthalein tests (a dye retention test). The results of these two tests were not comparable; they were probably supplementary and not indicative of a single functional disturbance. Similar results were obtained by using these tests on clinical material. Smith et al. (1940) studied liver function in cats and rabbits experimentally poisoned with selenium and used a slight modification of the technique described by Watson (1936). No marked abnormality was noted, but it was concluded that the mild type of hepatic damage in these animals did not cause the excretion of urobilinogen in urine.

Gornall & Bardawill did not find the urobilinogen test of much use in dogs. Using Watson's technique they found difficulty in minimising the colour produced by non-urobilinogen substances. Even when time and dilution factors were rigidly controlled strong positive tests were sometimes given by urine showing only traces of urobilinogen when subjected to the longer quantitative method of Schwartz et al. (1944). It was thought this might be due to the presence of dextro-urobilinogen. In the urine of normal dogs "apparent" urobilinogen levels of 0–2 mg.% were noted. During CCl_4 administration this value increased in some cases and decreased considerably in others. This apparent reduction might have been due to depression of bacterial activity in the intestines, and caution was advised in the interpretation of results obtained from patients receiving medication which might affect the intestinal bacterial flora.

Cornelius explained that the low renal threshold for bile pigments in dogs with biliary obstruction permitted accumulation of high concentrations of the interfering bilirubin glucuronide. Diuresis in chronic interstitial nephritis could continually dilute the urine urobilinogen below the sensitivity of qualitative tests. Changes in specific gravity of urine in order to maintain water balance would vary the urobilinogen concentration considerably, and it was nearly impossible to procure 24-hour urine samples for quantitative estimation of urobilinogen. In obstructive jaundice a decrease in urinary pigment might be expected but diurnal variations were common and diagnosis should not be based on the results of a single examination. In both haemolytic and hepatocellular jaundices increases of urinary urobilinogen occurred. For accurate quantitative estimations the technique of Watson et al. (1944) was best. This involved preliminary reduction of urobilinogen to urobilin by addition of ferrous hydroxide.

Svirbely et al. did not find the urobilinogen test as sensitive or reliable as the BSP test. The time required after liver damage had been produced by xylidine before large amounts of urobilinogen were excreted varied considerably. Excretion might increase in one or two days and then return to normal. Useful information could only be obtained by daily analysis of urine. Ott noted that urobilinogen decreased in obstructive jaundice and often increased in haemolytic and hepatocellular jaundice. Bloom (1957) reported similar findings. It is interesting to note that Karsai, using the technique of Wallace & Diamond, found that positive tests for urinary urobilinogen closely followed the increase of bilirubin in the blood.

Icteric index. The icteric index is said to measure the degree of jaundice by measuring the intensity of the yellow colour of the serum. Saline is added to 1 ml. of serum until it matches a standard solution of 1 in 10,000 potassium dichromate. The volume of saline added in ml. gives the icteric index.

Brouwers noted that in horses and dogs this was not a reliable test, as false positives could be caused by lipochromes. Bloom, however, stated that it was sufficiently accurate for practical purposes. He gave indices of 2–5 for normal dogs, 6–15 for latent jaundice and exceeding 15 for clinically evident cases of jaundice. Svirbely et al. found that the index only rose when dogs became moribund. Hoerlein et al. noted that both the icteric index and van den Bergh's test might be normal in some early forms of liver disease but in two cases of leptospiral jaundice icteric indices of 100 were found.

2. Tests concerned with administration of bilirubin and certain dyes.

The next series of tests are also associated with bile excretion. They involve intravenous injection of bilirubin itself or of various dyes which are excreted in bile and subsequent estimation of these substances in the blood at stated intervals. These dyes include phenoltetrachlorophthalein, phenoltetraiodophthalein (isodeikon), phenoltetrabromophthalein sodium sulphonate (bromsulphalein or BSP) and tetrachloro-p-tetraiodo (R) fluorescein (rose bengal).

Smith et al. used the bilirubin excretion test on normal cats and rabbits and on animals subjected to chronic selenium poisoning. 5 mg. freshly prepared 0.2% bilirubin in 2% sodium carbonate were injected intravenously. Blood sampling after five minutes and after two hours was found the most accurate method for comparative purposes. Average retention at two hours was 8% of that at five minutes. 15 cats showed a degree of retention while only seven had liver lesions. This did not seem to be a good correlation and it was assumed that bilirubin retention in cats was not necessarily associated with marked morphological changes.

Rosenthal & White (1924) had found in rabbits with liver damage caused by ligation of most of the liver that certain dyes, for example the chlorine, bromine and iodine compounds of phenol, cresol and resorcinolphthalein, indicated by their rate of disappearance from the bloodstream the degree of hepatic damage. Phenoltetrabromophthalein sodium sulphonate of BSP appeared to be the most suitable for this purpose.

Mills & Dragstedt (1938) compared the rates of excretion of bilirubin and BSP under various conditions and commented that while BSP and bilirubin were excreted almost entirely by the bile, both dyes left the blood stream at different rates and these were affected quantitatively and qualitatively by different procedures. Decrease in the rate of removal of intravenously injected BSP from the blood stream did not indicate the same pathological conditions as a similar change in the rate of excretion of bilirubin. The removal of BSP was apparently mediated by the reticulo-endothelial system and the rate of removal of this dye reflected the functional state of these cells. On the other hand, removal of

bilirubin appeared to be mainly related to and dependent on its excretion in the bile, and it was considered that its rate of removal reflected the state of parenchymal cells of the liver.

Pratt, Vanlandingham, Talley, Nelson & Johnson (1933) carried out BSP tests on one group of normal dogs and another group in which the portal vein and inferior vena cava had been anastomosed artificially. The authors concluded that BSP was eliminated specifically by the liver and that its estimation was a delicate and effective method for the study of hepatic function.

Brauer & Pessotti (1950) used a continuous infusion technique to determine the relationship between infusion rate, plasma BSP and bile BSP concentrations. They concluded that 77–89% BSP leaving the blood was extracted by the liver.

Smith et al. used intravenously injected BSP and rose bengal to estimate liver damage in selenium poisoning. The amount of residual rose bengal was variable at 15 minutes, more uniform at 30 minutes and too small to measure at 60 minutes. All of 16 experimentally poisoned cats showed a degree of retention at 30 minutes. Normal retention was 8%, whereas in poisoned cats the average was 22. These authors considered that rose bengal retention was a very sensitive test which could demonstrate slight dysfunction in cases where only lymphocytic infiltration could be seen microscopically.

Svirbely et al. used bilirubin, BSP and rose bengal retention tests on dogs experimentally poisoned with xylidine. BSP retention of up to 10% at 30 minutes could be taken as normal, 10–15% was suggestive of liver damage and over 15% was definitely abnormal. The results with rose bengal closely paralleled those with BSP, but values obtained from normal dogs varied because it was difficult to measure dye concentration in the presence of varying serum turbidity, and it was necessary to take initial blood samples to act as blanks. Retentions up to 15% were considered normal, 15–20% as suggestive of liver damage and above 20% as definitely abnormal. The bilirubin retention test was less sensitive and more erratic than the dye tests, abnormal retentions not being observed until seven days after the dye tests indicated hepatic disorder. Therefore it seems that the dye tests are probably better for routine use by veterinary surgeons.

Drill & Ivy (1944) studied dogs experimentally poisoned with CCl_4 and performed BSP tests using doses of 5 mg./kg. body weight. Using lower standards they found that the test was very sensitive.

In a similar study on dogs Gornall & Bardawill used 10 mg./kg. and collected blood samples after 15 minutes. They agreed that this test was very satisfactory.

The BSP test has also been used to indicate hepatic dysfunction in clinical cases. Hoerlein & Greene demonstrated abnormal retention in 22 of 50 dogs and two cases of leptospiral jaundice showed 100% retention at 30 minutes. One dog with cirrhosis retained 10% at 30 minutes, 5% at 40 minutes and a trace at 60 minutes. Four cases had shown dye retention, while other tests had proved normal (icteric index 1–6, van den Bergh .1–.5 mg.% methylene blue 1–5 drops).

One experimentally poisoned dog which had shown an apparent

clinical recovery gave a nearly normal BSP test thereby indicating that the liver had returned to normal. This was proved at autopsy. Hoerlein & Greene noted that in bilirubinaemia BSP estimation was difficult because the dye reacted with serum to give a red colour.

Nielson (1952) used the BSP test and expressed his results as serum concentration of dye in mg./100 ml. rather than percentage of maximum concentration. Determinations on normal dogs gave results of 0.21 mg. after 30 minutes or 0.1 mg. after 60 minutes. BSP tests were carried out on 14 dogs with various disorders but no symptoms of liver damage; 10 dogs showed a retention 0.2–0.3 mg.% after 30 minutes and readings of 0.4–0.5 mg.% were judged as slightly increased retention. Another 17 dogs with liver disorders showed retention of 0.7–7.3 mg.% showing that 7–73% of injected dyestuff was circulating after 30 minutes. These dogs subsequently died or were destroyed and there was a good relationship between the intensity of liver damage and dye retention.

Cornelius stated that the BSP test appeared to be nearly perfect for detecting latent liver damage, but unfortunately it was not possible to correlate the cellular retention with a specific type of lesion. There were many contra-indications to the use of BSP: the sensitivity of the test was such that a degree of retention could be found in conditions such as fever. Use of cholates would interfere with the test since competition for excretion into the bile existed between bilirubin, BSP and cholate, with BSP ranking the lowest. The BSP test could not be used in the differential diagnosis of icterus, since the mechanical effects of biliary obstruction would automatically produce retention. In addition alkaline serum samples containing bile pigments exhibited a red colour which prevented the quantitative estimation of the violet BSP. Cornelius advised the use of doses of 10 mg. BSP per kg. body weight.

Dye retention tests have also been used in large animals. Hornby (1924) found that 5 mg./kg. of phenoltetrachlorphthalein did not give any indication of subsequently proven hepatic insufficiency in two cattle with suspected liver disorder and Garner (1952a) stated that the dye excretion tests were not reliable in cattle.

Robertson (1957) used BSP to measure liver dysfunction in ketosis in cows and found a depression in BSP clearance. Freese (1953) used BSP at 2 mg./kg. for assessing liver damage in diseases such as piroplasmosis, fascioliasis, food intoxications and metabolic disorders in cattle, and Vardiman (1953) compared biopsy results with the BSP test in the diagnosis of senecio poisoning. The test was considered too sensitive for practical use because it gave indications of liver damage which could not be substantiated by biopsy. Also it did not differentiate between hepatitis and cirrhosis so that it could not be considered a diagnostic test for senecio poisoning.

PART II. TESTS CONCERNED WITH METABOLISM

This part will deal with tests concerning protein metabolism, serum enzyme activity, carbohydrate metabolism and fat metabolism.

After breakdown of proteins in the stomach and intestines the resulting amino acids leave the intestinal mucosa and pass to the liver by

the hepatic portal system, where most are retained and are used either for synthesis of body proteins, or undergo deamination or transamination.

The liver is responsible for several special features of protein metabolism among which is the conversion of uric acid to allantoin by uricase. This reaction can be used to assess liver damage.

Various proteins are probably produced entirely by the liver and one of the most important of these is plasma albumin. In liver disease there is generally a decrease in plasma albumin and an increase in globulin.

1. Tests concerning protein metabolism.

A. Tests involving serum proteins

Electrophoretic separation. Campbell (1957) described the application of paper electrophoresis to normal domestic animals. He also noted that certain diseases presented a regular alteration of serum protein pattern irrespective of species, while diseases specific to one species produced their own characteristic changes and served as an aid to diagnosis. In hepatitis there was consistent increase in gamma globulin with a decrease in the albumin fraction; inconsistent small changes were sometimes found in the alpha and beta fractions. Carcinoma of the liver greatly increased gamma globulin and reduced alpha and beta fractions. While the severity of liver damage was broadly related to the increase in gamma globulin a prognosis could not be given on the finding alone and the clinical picture should always be taken into consideration.

Boguth (1954) stated that increased gamma globulin indicated inflammation and cirrhosis of the liver; he also found increased beta and gamma globulins in hepatitis and other liver damage in dogs and cattle and also in hyperplastic endometritis and pyometra in bitches.

De Wael (1956) compared the percentages of serum proteins found in hepatitis and cirrhosis and demonstrated that in both diseases the mean albumin percentage was lowered significantly, the alpha-1 globulin was practically unaffected; there was a significant relative increase of alpha-2 globulin in hepatitis (this occurred also in benign and malignant tumours). In both hepatitis and cirrhosis resolution was poor in the region of beta and gamma globulins and therefore it was necessary to use the sum of the beta-1 and beta-2 globulins in hepatitis and the sum of beta-1, beta-2 and gamma globulins in liver cirrhosis. In hepatitis the alpha-2 band was more pronounced, while in cirrhosis the beta and gamma globulin bands were strong and poorly resolved.

Vesselinovitch (1959) in an analysis of serum proteins of domestic animals by filter paper electrophoresis also produced a table of results for normal animals. He pointed out that qualitative change in proteins might not have physiological effects in spite of an increase in amount, but qualitatively unchanged but quantitatively unbalanced serum proteins might be incapable of performing the normal functions. Ebel (1954) studied infectious hepatitis in nine dogs and observed either slight hyperalbuminaemia or hypoalbuminaemia in association with a rise in the alpha-2 and gamma globulins. Campbell, however, observed a rise in the beta

globulin fraction in one dog with hepatitis but it was not certain if this had a negative leptospira titre. Leptospirosis may be associated with a rise in either beta or gamma globulins so that concomitant pathological processes could influence the serum protein picture. Vesselinovitch induced fatty degeneration and centrilobular necrosis of the liver by ligating the hepatic artery. Serum proteins had been analysed before and after the operation. At the end of the experiment the dog had been destroyed and its tissues examined histologically. The albumin fraction decreased from the second day of operation while the alpha globulin increased.

Simple fractionation. This test was used by Gornall & Bardawill to fractionate serum proteins into albumin and total globulin. The technique of Howe (1921) was used first, but this was later superseded by that of Majoor (1946). These methods showed the protein pattern in dogs to be similar to that of human beings.

Dogs poisoned with CCl_4 showed a rise in euglobulin, with a less rapid decrease in albumin. Pseudoglobulin also tended to decrease slightly. The albumin : euglobulin ratio decreased during and sometimes after the administration of CCl_4. The ratio was always low when the BSP and alkaline phosphatase values were high and dogs with the lowest ratio were in the poorest state clinically. Cornelius gave normal values for total serum proteins as 5-7 g.% and stated that while no alteration in serum proteins was entirely specific for liver damage, a combination of low albumin and high globulin was very typical. The rapid fractionation method of Wolfson et al. (1948) was recommended.

Empirical turbidity and flocculation tests on serum. There are many of these at present in use in human medicine. The thymol turbidity test is one in common use and many theories have been advanced to explain its mechanism (Maclagan, 1944a, b, 1947, 1948, 1957; Cohen & Thompson, 1947; Kunkel & Hoagland, 1947; Ducci, 1951 and Popper, 1956). For example the last author considered that the changes in gamma globulin were not entirely responsible for the turbidity and flocculation. three factors which might be concerned with the reaction were change of serum albumin, depression of turbidity or flocculation by regurgitation of biliary material and change in gamma globulin concentration as measured by turbidity.

Maclagan (1948) gives the following list of tests:-

Formol Gel	(Gaté & Papacostas, 1920)
Takata-Ara	(Takata & Ara, 1926; Jezler, 1930)
Weltmann Coagulation Band	(Weltmann, 1930)
Cephalin Cholesterol	(Hanger, 1939)
Colloidal Gold	(Gray, 1940; Maclagan, 1944 a)
Thymol turbidity	(Maclagan, 1944 b)
Thymol flocculation	(Neefe, 1946 – Maclagan, 1947)
Cadmium Sulphate	(Wunderley & Wuhrmann, 1945)
Sharlach Red	(Maizels, 1946)
Zinc Sulphate	(Kunkel, 1947)

The Takata-Ara test is useful for the diagnosis of cirrhosis although

some false positives may occasionally be given. Maclagan (1956) described a new technique for this test, involving the use of a phosphate buffer to standardize pH.

Turning now to the application of these tests to veterinary medicine, Gornall & Bardawill used the thymol turbidity test. In normal dogs the turbidity readings varied from 0.2–1.2 units, but where liver injury had been produced by CCl_4 there was a tendency for turbidity to increase to above two units and in one case a value of four was obtained.

Karsai (1954) also used this test on clinical and experimental cases. Turbidity was measured by a photometer. In normal dogs photometric transmission varied between 69 and 89%. In clinical cases the transmission decreased significantly only in acute change: in no case did the depression reach the 15–20 value recorded in human beings with liver disease. In cirrhotic conditions values lay between 30 and 40 (or higher) whereas in dogs showing acute severe liver changes they were between 22 and 36. In Karsai's experience serum taken a few hours before death was often negative or less positive than in the early stages of disease.

Gornall & Bardawill also made use of the zinc sulphate test described by Kunkel and the sodium sulphite test devised by Dauphinée & Campbell (1948). Neither test was as sensitive in dogs as in man.

Karsai (1954) used modifications of the Takata-Ara test described by Jezler (1930) and Ucko (1936) but the specificity of Ucko's test seemed very doubtful and it was of diagnostic value only when a flaked precipitate was produced in all tubes immediately; but this reaction was given by some perfectly healthy dogs.

Jezler's test rarely gave positive results. It was negative in most cases associated with mild changes and sometimes failed to give a positive reaction in clinical hepatitis, but fairly positive results were obtained in proven or suspected cirrhosis.

Ferreira Neto (1958) considered the Takata-Ara reaction helpful in diagnosing chronic liver disease in dogs.

Cornelius stated that false positives might be given by most of the turbidometric or flocculation tests in dogs. This was because the albumin:globulin ratio was normally 0.7 in the dog, while it was 1–1.5 in human beings.

He stated that change in serum proteins was most commonly associated with hypertension caused by chronic hepatic changes. Since only 14% of the total canine serum proteins were changed daily, some time might elapse before tests depending on protein alteration became useful. Both low albumin and high globulin levels could be found in conditions other than liver disorder, so a careful interpretation of results was always needed.

Svirbely et al. found that methods of determining the albumin : globulin ratio were lengthy and involved and the results were of no diagnostic value in dogs with liver injury.

Darraspen & Florio (1951) concluded that flocculation tests were unreliable in the dog and probably depended on a more complex mechanism than in human beings.

B. Tests involving metabolic degradation of proteins

Uric acid estimation. In cases of hepatic dysfunction the blood levels of uric acid are raised. Techniques for estimation of uric acid are somewhat complicated (Brown, 1945; Bidmead, 1951).

Ott stated that blood levels were high in hepatocellular jaundice but not in obstructive or haemolytic jaundice in dogs. Bloom gave the normal blood level in dogs as 0.1–1 mg.%. Cornelius agreed with this figure and described the test as a sensitive and accurate indicator of liver disease. Ferreira Neto stated that it was one of the most practical liver function tests.

It is interesting to note that in Dalmatians although uricase is found in the liver in normal amounts only a small amount of the total proteins are excreted as allantoin and the majority are excreted as uric acid. A possible explanation is that the Damatian has a low renal threshold for uric acid, which passes into the urine before it has been oxidized.

Amino acid tolerance tests. These estimate the ability of the liver to deaminate amino-acids. Gornall & Bardawill used a tyrosine tolerance test to detect liver damage in dogs given carbon tetrachloride. Tyrosine was given orally to normal dogs and to dogs with liver damage and at intervals thereafter blood was sampled for tyrosine estimations.

Fasting tyrosine values in six normal dogs ranged from 1.1–1.9 mg.%. In two of three dogs which had been given CCl_4 the fasting value 48 hours later was above 2 mg.%, but the third was normal. The test appeared to show some liver damage in dogs but there were insufficient data for an estimate of its reliability.

The rate of removal of arginine was also investigated. A solution of 1.5 g. of l-arginine hydrochloride in 10 ml. of water was neutralized and injected intravenously; blood levels were determined over four hours. The results in two normal dogs were compared with those in three dogs with damaged livers. There was no significant difference in the rates of removal in both groups but four hours after the injection dogs with impaired liver function had slightly higher arginine levels than normal.

Goettsch et al. used plasma amino-acid retention in three hypoproteinaemic dogs. They noted that impairment of liver function could be detected as early as 16 days after a low protein diet was instituted.

Doggart, McCredie & Welbourn (1958) used paper chromatography to study amino-acid excretion in urine of dogs. Amino-acid excretion in dogs with cirrhosis increased only when there was severe liver damage. When damage was less severe there was selective increase in taurine and glutamine in the urine.

Karsai noted that deamination was incomplete in liver dysfunction, consequently the level of amino-acids in the blood was raised and a considerable proportion was excreted in the urine. Increase of tyrosine and leucine crystals in urine indicated not only upset liver function, but also extensive destruction of liver cells. Abundance of amino-acids could be demonstrated with Millon's reaction. In normal dogs this test was always negative. In clinical cases it was rarely positive unless liver

damage was very severe. The test was valuable not so much for early recognition of liver disease as for indication of its severity.

C. Transamination tests

Transamination involves the exchange of the alpha-amino group of an amino-acid for the keto group of an alpha-keto-acid, resulting in the synthesis of a second alpha-amino-acid and a new alpha-keto-acid. These reactions take place in the presence of the enzymes Serum Glutamic Oxaloacetic Transaminase (SGOT), and Serum Glutamic Pyruvic Transaminase (SGPT). In liver dysfunction the amount of these enzymes in serum is greatly increased and hence their estimation can be used as a test of liver function. They can be measured by colorimetry, spectrophotometry, or chromatography; these methods have been discussed by Wroblewski (1957). In man SGPT is probably a better indicator of liver cell function than SGOT as it is more selectively concentrated in liver cell tissue.

Cornelius, Bishop, Switzer & Rhode (1959) studied: (1) which tissues could release significant quantities of SGOT and SGPT due to pathological alteration of blood-cell barriers; (2) normal SGOT and SGPT activities in horses, cattle, swine, dogs and fowls; (3) enzyme activities following carbon tetrachloride poisoning in horses, cattle, swine and dogs.

The colorimetric method was used and enzyme activity was expressed as μg. of pyruvic acid liberated in 20 minutes at 25° C. per ml. serum or mg. of dry tissue.

SGOT activities were higher in horses than in the other mammals studied. SGPT activities of various mammals were less than 30 μg. of pyruvate per ml. Young calves less than a month old had lower activities of both enzymes. Considerable SGOT was found in all tissues analyzed in the various species, the greatest activity being in heart, muscle, liver and kidney and least in lung, spleen and intestine.

Significant elevations in SGOT activities of horses, cattle, pigs, dogs and chickens occurred in necrosis of liver, heart, kidney and skeletal muscle.

Following CCl_4 administration and subsequent hepatic necrosis all domestic animals showed a marked increase of SGOT activity which appeared to result from escape of enzyme to serum from injured hepatic cells. Inability of liver cells to excrete transaminase into the biliary canaliculi, or its liberation from tissues other than the liver, could also affect SGOT activity.

The relatively low activities of SGPT in horses, cattle and pigs following the administration of CCl_4 was consistent with the absence of appreciable SGPT activity in the livers of these animals. Dogs, however, showed much more SGPT activity in normal liver tissue than the other domestic animals and after CCl_4 poisoning the serum content of this enzyme was very high, up to 550 times the normal value. Therefore SGPT activity could be recommended in the differential diagnoses of liver disease in the dog. However, the possibility of minor elevations from myocardial and renal necrosis should not be overlooked as the heart and kidneys contain SGPT activity amounting to a quarter of the quantity in

liver. Determination of SGPT activity appeared to be useless for diagnosing hepatic necrosis in horses, cattle and swine.

2. Tests involving serum enzyme activities.

This group includes estimation of serum alkaline phosphatase (SAP), prothrombin time and serum cholinesterase (SChE). Tests based on transaminase enzymes have been described in the preceding section.

Alkaline phosphatase test in serum. The function of alkaline phosphatase is to catalyse the "splitting off" of phosphoric acid from certain monophosphoric esters. In many cases of liver dysfunction the serum content of alkaline phosphatase (SAP) is greatly increased and it can be used to test liver function. This enzyme shows maximum activity at pH 10 and the methods most commonly used for its estimation are those of King & Armstrong (1934) and King, Haslewood & Delory (1937). The methods of Bodansky (1933, 1937) and Shinowara, Jones & Reinhart (1942) can also be used.

The origin of the enzyme is not known and neither is the mechanism which increases SAP in jaundice.

Armstrong, King & Harris (1934) experimentally obstructed the common bile duct in 19 dogs and afterwards determined SAP and serum bilirubin daily. In every case SAP progressively increased reaching 30–100 times the initial amount in six days. In two dogs the obstruction was later relieved and during recovery phosphatase fell to the original concentration. In experimental haemolytic jaundice in three dogs and haemolytic jaundice in two human beings there was no appreciable rise in serum phosphatase.

Freeman, Chen & Ivy (1938) compared the results of SAP estimations in four groups of dogs. The first group had leptospiral jaundice; all had increased phosphatase activity but the values were not as high as in puppies with experimental obstruction of the common bile duct. The puppies with leptospirosis also showed an increased icteric index and gave a direct van den Bergh reaction. The average SAP before injection was 5.5 King-Armstrong units and this increased to 20.4 units after leptospirosis became evident. Histological examination revealed diffuse hepatitis without obstruction to the extra-hepatic biliary system. The second group consisted of dogs given CCl_4. Increase in SAP was noticed soon after CCl_4 administration began but it returned to normal after 100 days. The third group had experimentally produced bile fistulae and some were apparently healthy while others were jaundiced. All had elevated SAP with a great range of values. The jaundice-free animals ate well and had lower SAP values than the jaundiced. The fourth group of dogs had experimentally produced obstruction to the common bile duct. All these showed a marked elevation of SAP with values up to 100 units per 100 ml. of serum.

Freeman & Chen (1938) also considered that estimation of SAP was a delicate test of liver function.

Drill & Ivy compared the BSP, SAP, prothrombin time and intravenous galactose tests in detecting hepatic damage produced by CCl_4.

They concluded that the SAP test was nearly as sensitive as the BSP but the rise in SAP was greater in some dogs than in others and the degree of change did not always parallel the changes in BSP.

Drill, Annegers, Snapp & Ivy (1944) demonstrated that dogs with biliary fistulae had abnormal hepatic function and in most of them BSP and SAP estimations gave simultaneous evidence of hepatic damage.

Svirbely et al. compared various tests in detecting hepatic damage produced by xylidine and found that the SAP test was useful. Values above four Bodansky units per 100 ml. were considered abnormal. Moderate rises were observed during the first week of poisoning and these became high by the fourth week, but the test was not as conclusive as the dye tests. Gornall & Bardawill, using the technique of Jones & Reinhart (1942) on dogs poisoned with CCl_4, found that SAP activity rose as much for hepatocellular damage as for biliary obstruction. They noted a rough correlation between BSP retention and SAP. Bloom stated that 3–6 Bodansky units of SAP per 100 ml. was normal for dogs and 6–10 units indicated non-obstructive jaundice; values greater than 10 units were indicative of obstructive jaundice. Cornelius questioned the significance of SAP as a test for liver function in dogs, as any syndrome accompanied by secondary hyperparathyroidism might increase SAP.

Garner (1952b) found the normal range of SAP in 294 zebu cattle varied from 5–242 King-Armstrong units. This wide range prohibited its use as an indicator of hepatic dysfunction.

Prothrombin time. The liver is thought to be responsible for the production of prothrombin and also of fibrinogen. Both are concerned with blood clotting and in liver disorders this function may well be defective. Two causes for deficient production of prothrombin are direct damage to the polygonal cells and failure of vitamin K absorption in the absence of bile. Hepatitis does not generally interfere with fibrinogen production. Deficiency of prothrombin will lead to a defective blood clotting which can be detected by determining prothrombin time, defined as the time required for clotting to take place in oxalated plasma to which optimum amounts of thromboplastin and calcium have been added.

Gornall & Bardawill modified the technique described by Quick (1936) on blood from normal dogs and dogs poisoned with CCl_4. Clot formation was determined by gently sweeping the mixture with a wire loop, the first appearance of fibrin strands being taken as the end point. Normal dog plasma had a prothrombin time of 7.4–8.6 seconds. Five dogs had increased prothrombin time from 11–24 seconds after oral administration of two doses of CCl_4, but they returned to normal within ten days. Six dogs which had received CCl_4 for several months had normal prothrombin levels, even though their blood seemed to have a definitely prolonged spontaneous clotting time. Gornall & Bardawill concluded that this test might indicate liver damage during the early stages.

Drill & Ivy found the prothrombin time not as reliable or sensitive as the BSP or SAP tests. They pointed out that the conversion rate of prothrombin was faster in dogs than in man and therefore an increase in the rate of conversion might mask a deficiency. In dogs

poisoned with CCl_4 hepatic damage was determined in only one dog as early as it was detected with BSP and in four dogs normal prothrombin time was maintained.

Svirbely et al. used the test on dogs with hepatic damage caused by xylidine. A ratio of prothrombin time of control plasma to prothrombin time of an unknown sample greater than 1.2 was abnormal. Generally the readings increased with increasing retention of BSP.

Cornelius stated that low plasma prothrombin could occur in advanced diffuse hepatic fibrosis. In scarring of the liver following massive necrosis there was generally sufficient regenerated hepatic tissue to carry on normal prothrombin production. When clotting was delayed in the presence of icterus, a 50 mg. injection of vitamin K decreased the coagulation time if simple extra-hepatic obstruction existed. Coagulation time could be rapidly and simply determined by the use of capillary glass tubes 1 mm. in diameter. The buccal surface of the upper lip of the animal was punctured and a drop of fresh blood drawn into the tube by capillary attraction. Parts of the tube were broken at 15-second intervals and examined for the presence of fibrin, which normally occurred by 1–1.5 minutes. Coagulation times of two minutes and more were abnormal.

Cholinesterase in serum. Serum cholinesterase (SChE) is thought to be a mucoprotein synthesized by the liver. In liver diseases its production is likely to be defective and therefore estimation of the enzyme can be a useful liver function test. It has been widely used in human medicine and is said to reflect very accurately the nutritional state of the liver. This enzyme, besides hydrolyzing acetylcholine into choline and acetic acid, can also hydrolize other esters, for example, fats.

The most popular method for determination of SChE in human medicine is to measure depression of pH of a buffered substrate caused by acetic acid produced by enzyme action. The test has not been widely used in veterinary medicine. Ferreira Neto stated that he found low SChE activity in two dogs with liver disease and high activity in another.

3. Tests concerned with carbohydrate metabolism.

Various tests of liver function are based on administration of different sugars and their subsequent estimation in the blood. In human medicine the galactose tests appear to be the most practical and to give the most sensitive index of liver function. Utilization of galactose appears to take place only in the liver and it is independent of the action of insulin; its metabolism seems to impose maximum strain on the liver cell.

Galactose tolerance test. Drill & Ivy used the intravenous galactose test of Bassett, Althausen & Coltrin (1941) to detect liver damage in dogs poisoned with CCl_4. The results were difficult to interpret and generally damage was not detected as early as with the prothrombin time test.

Colcher, Patek & Kendall (1946) employed a "galactose removal

constant" and claimed that it was as sensitive as the BSP test in human beings. Gornall & Bardawill found no significant difference between the galactose removal constants obtained for normal and abnormal dogs.

Markiewicz (1956) used the galactose test on normal dogs and dogs poisoned with thallium. In poisoned dogs the pre-injection concentration of blood sugar was regained much more slowly. In normal dogs the pre-injection concentration was reached in one hour, while in dogs with impaired liver function it took two hours; the concentration of reducing substance 30 minutes after injection was 30% greater than before injection and even after 90 minutes it was higher.

Mazusczak & Tonicki (1956) used a modification of the chromatographic galactose test on dogs. This was stated to be easy to perform and gave better results than other techniques.

Garner (1952c) tried the galactose tolerance test to estimate liver damage in cattle, using the adaptation of King & Aitken (1940). Galactose was given intravenously as a 50% solution at the rate of 0.5 g./kg. body weight to animals which had been fasted overnight.

The reduced galactose time observed in cattle with mild diffuse liver damage was striking but the shape of the galactose tolerance curve seemed to give a better indication of liver damage.

Adrenaline response test. Garner also used the adrenaline response test. He discussed the assumption that the rise in blood sugar following injection of adrenaline was related to the glycogen content of the liver. It had been believed that the rise in blood sugar resulted from accelerated hepatic glycogenolysis but Somogyi (1950) suggested that adrenaline probably depressed glucose assimilation.

Although there was a difference in the response to adrenaline of normal and diseased cattle in the small numbers examined, the results were variable and indecisive. But the initial rate of increase in blood glucose provided a fairly clear distinction between normal and diseased animals.

Garner concluded that with suitable modifications the galactose tolerance and the adrenaline response tests could both be used to detect hepatic dysfunction in cattle.

4. Tests concerned with fat metabolism.

Estimations of total cholesterol and of the ratio free cholesterol to esterified cholesterol can be used as liver function tests. In obstructive jaundice in human beings there is an increase of total cholesterol in serum, probably due to over-production by the liver and not merely to retention of cholesterol normally excreted into bile (Byers, Friedman & Michaelis, 1951). When obstruction is caused by carcinoma the resultant malnutrition will cause a considerable fall in cholesterol value (Sherlock).

Hepatocellular jaundice depresses esterification, so the ratio of free to esterified cholesterol is higher than normal.

The Liebermann-Burchardt reaction is generally used to estimate total cholesterol.

Cornelius stated that total cholesterol in canine serum ranged

between 125 and 250 mg.%, of which 70% was normally esterified. In liver diseases certain changes occurred but it was difficult to draw conclusions from them. While imperfect liver function caused a fall in cholesterol esters, it could also prevent rise in total cholesterol such as occurs in uncomplicated obstructive jaundice. In extra-hepatic biliary obstruction the total cholesterol level increased without change in the percentage of esters present. A decreasing ester level suggested damage to liver cells. High blood cholesterol occurred in other diseases in dogs such as hypothyroidism, diabetes mellitus and advanced nephrosis. Clarification of this test must evidently await future investigations in lipide metabolism. Bloom gave total cholesterol values as 140–210 mg.%, 60–80% being in the esterified form. He stated that definite variations occurred in liver disease.

Darraspen & Florio found that the ratio free cholesterol to esterified cholesterol gave a far better indication of liver function than turbidity tests.

PART III. TESTS BASED ON DETOXICATION MECHANISMS OF THE LIVER

The liver deals with toxic substances by conjugation and by destruction. Conjugation is effected by amino-acids, their derivatives, and other substances such as glucuronic acid, which combine with the toxic agent to produce a less toxic substance.

Hippuric acid synthesis. Bloom considered this test to be useless in dogs as in this species hippuric acid is synthesized only in the kidney. In rabbits Aldersburg & Minibeck (1936) showed that the test detected periportal fibrosis and infiltration but was less effective in parenchymatous necrosis. Smith et al. used it to determine liver function in normal and selenium-poisoned rabbits. Elimination of hippuric acid was studied following injection of benzoic acid with or without glycine. In normal rabbits 57% of the benzoic acid administered was excreted as hippuric acid during the first four hours. In poisoned animals the average excretion for the same period was 40%. Glycine did not materially increase the output of hippuric acid in 22 hours, but it increased the rate of conjugation in the first two hours.

Hippuric acid excretion usually parallelled retention of intravenously injected rose bengal, but rose bengal retention was more often positive and was more consistent with the histological finding.

Chloral hydrate excretion. Mukerji & Ghose (1940a) discussed the urinary clearance of free chloral in normal dogs and in dogs with liver damage caused by CCl_4. They determined chloral by the method of Friedman & Calderone (1934). After administration of 200 mg./kg. of chloral hydrate normal dogs excreted only 0–6 mg. in 24 hours but in dogs with liver damage there was always a significant increase in excretion. This increase was seen in the dogs

given only two small doses of CCl_4 as well as in those whose livers were presumed to be badly damaged. The test appeared to be very sensitive. For clinical estimations smaller doses of chloral hydrate (100 mg./kg.) were recommended. It was concluded that this drug was apparently not as toxic as had been assumed.

Glucuronic acid excretion. Toxic drugs such as chloral are rendered innocuous in the liver by conjugation with glucuronic acid. Mukerji & Ghose (1940b) administered chloral to rabbits and dogs by stomach tube in increasing doses until the urinary excretion of conjugated glucuronic acid increased. Experimental liver damage was then produced by repeated doses of CCl_4, after which chloral was again administered. Estimations were carried out by Salt's modification of the napthoresorcinol test. In dogs with chronic liver damage there was an initial phase of irregular excretion followed by a marked increase which was maintained for a fair time, but after 40–50 administrations of CCl_4 a period of decreased excretion became evident. There was a difference between rabbits and dogs in the normal amount of glucuronic acid excreted and in the dose of chloral required to increase excretion. In rabbits detoxication by oxidation and reduction was probably more important than conjugation.

DISCUSSION

This review has shown that the assessment of liver function in the domestic animal is a most complex procedure. Limited work has been carried out on actual clinical cases, the majority of workers having relied on animals with liver damage caused by experimental poisoning.

One has to bear in mind the amazing powers of healing and regeneration of liver. Himsworth (1947) stated that in zonal necrosis three quarters of every liver lobule may be dead and yet reconstitution is possible within a fortnight. It is difficult to detect biochemically the complex cellular reactions which take place.

Klatskin (1948) commenting on tests of liver function in human medicine stated that the tests were called upon to answer three questions. was liver disease present; what was the nature of the disease. what course was established liver disease taking.

The first question could generally be answered by liver function tests, but the second was unfortunately not as simple. Tests were confirmatory rather than diagnostic and no one test could distinguish the many known types of parenchymal damage. It was possible to distinguish between haemolytic and hepatic jaundice, but it was doubtful whether any test would differentiate between all the cases of parenchymatous and obstructive jaundice, since obstructive lesions were sooner or later complicated by parenchymal damage. Serial estimations generally served to establish the course of liver disease.

Popper et al. (1949) discussed the relationship between results of liver function tests and morphological changes in liver tissue in human beings. For instance, they established that there is significant correlation between diffuse liver cell damage and bromsulphalein retention,

cephalin cholesterol, and thymol turbidity tests. They described the results of applying various tests to different forms of hepatitis and cirrhosis.

It seems that in veterinary medicine there remains much work to be done in the selection of a suitable range of liver function tests, preferably ones which can be performed without expensive laboratory equipment and which are not too time consuming to be used by the busy practitioner. If this work could then be extended to indicate the results obtained when these tests were applied to animals suffering from different forms of liver disorder, a definite step forward would have been taken in the field of veterinary diagnosis.

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Some Aspects of Non-specific Resistance to Infectious Diseases

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The specific immunity which follows recovery from most infectious diseases has been the subject of continuous study since the recognition that such diseases are caused by parasitic invasion of the tissues. These labours have produced a considerable knowledge of antigen–antibody reactions and led to successful methods of prophylaxis against the most serious infectious diseases. Although the principles involved are general and will doubtless be extended to a wider range of diseases, certain diseases present difficulties, especially those whose causal virus has multiple antigenic types, as is the case with foot and mouth disease; African horsesickness; bluetongue and influenza. In such cases, the short time available to produce and inoculate a vaccine of the correct type to prevent an impending epidemic must always render the outcome uncertain. A difficulty arises also with the less serious diseases. They are too numerous and often too ill-defined for prevention by specific immunization to be practicable. These limitations to the general usefulness of the classical methods of prophylaxis have helped to stimulate search for methods applicable to the diseases in which the classical methods are unsuitable. In general, recovery from an infectious disease results in a level of immunity sufficient to resist infection with doses of the aetiological agent far in excess of those encountered under natural conditions. However, if animals are inoculated with small doses of infectious agents, doses more comparable with those from which natural infections arise, only a proportion are infected and only a proportion of the infected become seriously ill. Investigations to discover the mechanisms in animals which determine their resistance to pathogenic agents in the absence of any specific immunity offer some hope towards finding a practical way to lessen the consequences of such diseases. Genetic constitution and such environmental factors as temperature and diet obviously influence resistance. However, such diverse factors probably produce their effects by direct action upon relatively few of the animal systems. The reticulo-endothelial system (r.e.s.) might be suspected to have an important function in this respect. The capacity of the phagocytic cells of the lymph nodes, spleen and liver — the reticulo-endothelial system (r.e.s.) — to clear from the blood-stream particles ranging in size from macro-molecules to those larger than bacteria has been intensively studied in recent years (see, for example, Physiopathology of the Reticulo-Endothelial System; A Symposium. 1957. Oxford: Blackwell Ltd.). These studies have provided the means to investigate the role of the r.e.s. in resistance to infections. Considerable work has

been done along these lines and it has become abundantly clear that the condition of such cells has an important influence upon resistance to disease. The influence is still imperfectly understood and some facets of the work even appear contradictory. This paper will give a résumé of those aspects of this work most likely to contribute towards an understanding of the function of the r.e.s. in resistance to disease.

Effect of substances of large particle size upon phagocytosis by the r.e.s. Methods to determine the rate of phagocytosis of particles from the blood-stream by cells of the r.e.s. and to express the results quantitatively have been devised (Benacerraf et al., 1957). Thus, it has become possible to determine the effect of inoculation of various substances upon the phagocytic activity of r.e. cells. Zymosan (a polysaccharide from yeast), Thorotrast (a colloidal suspension of thorium dioxide), saccharated iron oxide, all modify the phagocytic activity of the r.e.s. in a similar way. Endotoxins from Gram-negative bacteria and lipopolysaccharides in general produce the same sort of modification although they also produce other effects (Thomas, 1954). The substances which act upon the r.e.s. consist of suspensions of particles of a size to be phagocytosed by the cells of the r.e.s. The actual phagocytosis of such particles first lowers for a few hours the capacity of the r.e.s. for phagocytosis and then raises it to levels considerably in excess of the normal. The capacity for phagocytosis in fact reaches a maximum about 24–48 hours after inoculation of such substances and remains higher than normal for a week or more.

Properdin and resistance to disease. In 1954, Pillemer, Blum, Lepow, Ross, Todd and Wardlaw described the presence of a globulin fraction (properdin) in normal serum which possesses a bactericidal action in the presence of complement and which also has a lethal action upon some viruses (van Vunakis et al., 1956; Wedgwood et al., 1956). Pillemer & Ross (1955) showed that injection of zymosan or *Escherichia coli* cell walls into rats or mice causes a rapid fall in serum properdin followed by a rise above the normal level. The time scale of these changes of serum properdin closely conforms to that of the depression and elevation of phagocytosis. Finally, several groups of workers (Dubos & Schaedler, 1956; Landy, 1956; Rowley, 1956) showed that the substances which produce these similar patterns of variation in phagocytic activity and in level of serum properdin also produce the same pattern of variation in resistance to the lethal effects of Gram-negative bacteria, namely depression followed by elevation. At first it seemed a reasonable hypothesis that the changes of resistance to lethal infections were due to the changes of serum properdin (Rowley, 1956) but in the light of further work this view has been abandoned. Thus, Howard et al. (1958) found that although zymosan was effective in stimulating an increase in serum properdin, it did not raise resistance to infection with *E. coli* and that the development of a low properdin level during *E. coli* infection did not invariably lead to death. Ross (1958) found that a single injection of zymosan raised host resistance to infection with *Klebsiella pneumoniae* for periods up to three months despite the return of serum properdin to

normal levels and, furthermore, although passively conferred purified properdin gives protection, it also protects after destruction by heat of the bactericidal and other properties which characterize it as properdin (see Table).

Phagocytosis by the r.e.s. and resistance to bacterial infections. If, on the basis of such observations, it is considered that properdin plays at most a minor part in resistance to bacterial infection, the variations in resistance which follow the inoculation of bacterial endotoxin might be due to variations in the phagocytic activity of cells of the r.e.s. which, as we have noted, present a similar pattern of depression followed by elevation. However, this hypothesis in its simplest form does not accord with all the observations. It has for example already been observed that inoculation of zymosan fails to increase resistance to the lethal effect of E. coli and increases resistance to the lethal effect of K. pneumoniae for a period much in excess of that during which the phagocytic activity of the r.e.s. is enhanced (see Table). Similarly, Boenme & Dubos (1958) found that, whereas an inoculation of endotoxin increases resistance to infection with Mycobacterium fortuitum for many weeks, increased phagocytic power lasts for only eight days. The long duration of increased resistance can be explained by the supposition that primary inoculation of endotoxin not only increases the actual phagocytic power of the r.e.s. for about eight days but also raises its capacity to respond by increased phagocytosis when again stimulated by endotoxin or infection and that this raised capacity of response lasts for many weeks. The situation (see Table) that zymosan fails to increase resistance to E. coli infection is also encountered with Thorotrast which fails to increase resistance to Salmonella typhi-murium infection (Howard et al., 1958). S. typhi-murium organisms can multiply in the cells of the r.e.s. of mice and Howard et al. have suggested that this may account for anomalous behaviour of S. typhi-murium in mice inoculated with Thorotrast. Stimulation of phagocytosis could not be expected to increase resistance against organisms which multiply in the cells which have phagocytosed them. Since, unlike Thorotrast, endotoxin increases resistance to S. typhi-murium, it presumably raises the resistance of cells to the multiplication of the bacteria within them as well as increasing phagocytosis.

Effect of bacterial endotoxin upon resistance to ectromelia. Bacterial endotoxin in the form of 0.2 ml. of filtrate of growths of S. typhi-murium or 50 μg. of semi-purified endotoxin from Chromobacterium prodigiosum inoculated intraperitoneally into mice 24 hours before intraperitoneal inoculation of one million LD_{50} ectromelia virus increases the average survival time to about two and a half days compared with one and three-quarter days for mice which have received only the virus (Gledhill, (1959a). This result is essentially similar to that obtained when mice are infected with pathogenic bacteria and can be explained as due to the increased capacity of the r.e.s. of mice 24 hours after inoculation with endotoxin to clear the blood-stream of virus particles by phagocytosis and thereby to lessen the magnitude of primary infection of vital tissues such as the liver parenchyma. When smaller doses of virus are used, such as

TABLE

Effect upon infections of treatments which enhance phagocytosis by the r.e.s.

Substance	Duration of enhancement of phagocytosis by r.e.s.	Effect on resistance to infections: Increased resistance to infection with:	Effect on resistance to infections: Decreased resistance to infection with:	Reference
Endotoxin	About 1 week	E. coli (1 week)		Rowley (1956)
"	"	S. typhi-murium		Howard et al. (1958)
"	"	M. fortuitum (several weeks)		Boehme & Dubos (1958)
"	"	[Ectromelia – massive doses]	Ectromelia	Gledhill (1959a)
Endotoxin (very small doses)	"	Ectromelia		Gledhill (unpublished data)
Zymosan	"	K. pneumoniae (several weeks)		Ross (1958)
"			E. coli	Howard et al. (1958)
Thorotrast	"	E. coli	S. typhi-murium	"
Saccharated iron oxide	"	[Ectromelia – massive doses]	Ectromelia	Gledhill (1959a)

one thousand LD_{50}, mice pre-inoculated with endotoxin die in an average time of about five and three-quarter days compared with seven days for the control mice; in other words, pre-treatment with endotoxin shortens the survival time when mice are given smaller doses of virus. Pre-treatment with endotoxin similarly shortens the survival time of mice inoculated with one LD_{50} of virus and leads to a final mortality of more than 90% compared with 50% for the control mice. Since phagocytosis by r.e. cells is enhanced for at least a week after the inoculation of endotoxin, it is difficult to account for the decreased survival time in mice pre-inoculated with endotoxin. However, it is known that ectromelia virus multiplies in cells of the r.e.s. during the incubation period (Fenner, 1949). Presumably the cycle of events is as follows (Gledhill, 1959a). The inoculated virus is phagocytosed by r.e. cells and proceeds to multiply in them. Simultaneously, the processes leading to the production of antibody are initiated. In a mouse which develops the acute disease and dies, the multiplication of virus in the r.e.s. saturates its capacity to phagocytose virus particles; massive infection of vital structures, notably the liver parenchyma, follows and initiates the phase of manifest illness. In mice pre-treated with endotoxin the fact that illness develops sooner cannot be attributed either to decreased phagocytosis of virus particles or to delay in antibody production because pre-treatment with endotoxin enhances both these activities. It therefore appears likely to be due to increased production of virus, whereby saturation of the r.e.s. with virus occurs sooner. Since the metabolism of r.e. cells after stimulation by endotoxin pre-treatment is presumably greater than that of normal r.e. cells, it seems a reasonable assumption that the virus particles would grow more actively in such cells. This might lead to earlier saturation of the r.e.s. by virus even after allowance is made for increased phagocytosis and enhanced antibody production (Stevens & McKenna, 1958). The contrary result when the dose of ectromelia is massive, e.g. one million LD_{50}, is due, as we have explained, to lessened primary infection of vital tissues as a result of the increased speed with which the blood-stream of endotoxin pre-treated mice is cleared of virus particles by the r.e.s. during the first few minutes after infection. Similar results were obtained when mice pre-inoculated intravenously with several daily doses of saccharated iron oxide were substituted for those inoculated with endotoxin in these experiments and this supports interpretation of the results in terms of the action of endotoxin upon the r.e.s. This explanation of the increased pathogenicity of moderate and small doses of ectromelia for mice pre-treated with endotoxin is essentially the same as that put forward by Howard et al., as noted above, for the increased pathogenicity of S. typhi-murium for mice pre-treated with Thorotrast.

Effect of serum from mice reacting to adverse conditions upon the pathogenicity of ectromelia. Inoculation of bacterial endotoxin has a pyrogenic effect and two hours after inoculation of dogs and rabbits with endotoxin their blood serum contains a pyrogen distinct from endotoxin (Petersdorf & Bennett, 1957; Petersdorf et al., 1957; Atkins & Wood, 1955; King & Wood, 1958). It has been termed "endogenous pyrogen" and is believed to be produced by damaged cells, notably leucocytes. These

observations suggested that the inoculation into mice of serum from mice bled two hours after infection with endotoxin might increase their susceptibility to ectromelia virus in the same way as the inoculation of endotoxin. Experiments showed, in fact, that the inoculation of such sera increased the survival time compared with that of normal mice inoculated with about 10 LD_{50} of ectromelia virus (Gledhill, 1959b). A similar result was obtained when mice were inoculated with about 10 LD_{50} of the lethal mouse hepatitis virus/Eperythrozoon coccoides in place of ectromelia. (Gledhill et al., 1955). The sparing property of the serum resisted 56°C. for half an hour and this serves to distinguish it from properdin. More recently it has been shown to be filtrable but not dialysable and to act upon the mice rather than directly upon the virus (Gledhill, unpublished data). These results are considered to be due to the presence in the serum of a sparing substance produced by cells reacting to endotoxin and on this basis it was suggested that animals treated with other toxic agents might possess sparing substances in their sera. Mouse hepatitis virus (MHV), a virus of low pathogenicity in the absence of E. coccoides, was selected to test this hypothesis and it was found that the serum of mice two or three days after inoculation with MHV manifested a sparing effect upon ectromelia infection very similar to that of serum from mice reacting to endotoxin (Gledhill, 1959c). The effect was shown by sera which had been heated to inactivate MHV particles or filtered to remove them. Furthermore, it was observed that infection of mice with MHV interfered most markedly with the pathogenicity of ectromelia and it was suggested that this interference might be mediated by the continuous production of the sparing substance during several days. It is possible that other cases of viral interference result from the production by infection with one virus of substances which increase the resistance of cells to the pathogenic action of another virus. Unpublished experiments have shown that a similar sparing effect upon infections with ectromelia is manifested by the serum of mice inoculated intraperitoneally with ultraviolet inactivated influenza virus, and it may be that the production of the material termed interferon by cells of isolated pieces of chick chorioallantoic membrane treated with UV-inactivated influenza virus is a similar phenomenon (Isaacs & Lindenmann, 1957). Other unpublished experiments have shown that serum from mice kept for a few hours at 37° in still air also lessens the pathogenicity of ectromelia to about the same extent. Whether this observation bears any relationship to the sparing effect of low environmental temperature upon some virus diseases is unknown (for example, Parker & Thompson, 1942; Lwoff et al., 1959). These results support the hypothesis that mice reacting to a wide range of adverse conditions produce a substance or class of substances which tends to raise their resistance to the pathogenic action of ectromelia. Indeed, the production of such substances might appear to represent an important element of the mechanism of defence against infection and other adverse conditions.

Effect of small doses of endotoxin upon the course of ectromelia infection. Another interpretation of the results which follow the inoculation of endotoxin is possible. Recent experiments have shown that

when mice are inoculated with endotoxin in a dose of 10^{-2} to 10^{-3} of that used previously (see p.29) their resistance to the lethal effect of small doses of ectromelia is increased to about the same extent as by inoculation of serum from mice reacting to endotoxin. Furthermore, it would appear from the results obtained by Noyes et al. (1959) that the amount of inoculated endotoxin which would be present in the dose of serum (0.2 ml.) from mice reacting to endotoxin would be about 1/240th of the endotoxin inoculated. This would be sufficient to produce the sparing effect which such sera manifest and thereby explain the anomaly that, whereas mice inoculated with the dose of endotoxin employed become more susceptible to ectromelia or MHV/E. coccoides mixture, inoculation of the serum from such mice produces a sparing effect against these viruses. Wagner et al. (1959) have found that small doses of purified endotoxin from S. abortus-equi have a sparing effect against infections of mice with Eastern equine encephalomyelitis virus and encephalomyocarditis virus when both are given either by the intracerebral or intraperitoneal route. Thus, the inoculation of endotoxin in a suitable dose raises the resistance of mice to the pathogenic action of several different viruses. The effects may result from stimulation of phagocytosis by the r.e. cells (weak stimulation leading to increased phagocytosis of virus particles and stronger stimulation to both increased phagocytosis and increased multiplication of phagocytosed virus particles). Equally, the effects could be considered due to the stimulation by endotoxin of the production of substances which raise the resistance of cells to infection. This explanation would better accord with the elevation of resistance produced by sera from such other sources as mice maintained in a hot environment or inoculated with MHV or UV-inactivated influenza virus. It is perhaps significant that Likar et al. (1959) have observed that treatment of monkey kidney tissue cultures with endotoxins from E. coli and S. typhi-murium suppressed the growth of poliovirus. Moreover, it is possible that adverse circumstances lead to the endogenous production of polysaccharides of the same chemical class as endotoxins and that these raise resistance. This would be a situation difficult to establish experimentally and quite parallel to the irreversible shock syndrome which some workers have attributed to the absorption of endotoxin from bowel bacteria (Schweinburg et al., 1957) while others attribute it to endogenous polysaccharides produced under conditions of stress (Landy & Shear, 1957).

Augmentation of the toxicity of endotoxin. Mice inoculated with a sublethal dose of endotoxin become more susceptible to endotoxin for 6–12 hours and then become more resistant for about a week. On the basis of this cycle of susceptibility it is claimed that resistance to endotoxin depends upon the capacity of the r.e.s. to clear endotoxin from the bloodstream. Suter et al. (1958) showed that five days after intravenous vaccination of mice with B.C.G. they are about fifty times more susceptible to E. coli endotoxin than normal mice and the hypersusceptibility is still detectable 70 days after vaccination. They further showed that two injections of "cord factor", trehalose dimycolate, increase the susceptibility of mice about twenty-fivefold to endotoxin inoculated seven days

later. Halpern et al. (1958) obtained a similar result in B.C.G. vaccinated mice using killed S. typhi as source of endotoxin and emphasized that this observation contradicts the view that resistance to endotoxin depends upon the phagocytic activity of the r.e.s. Similarly, Benacerraf et al. (1959) showed that susceptibility to endotoxin was increased in mice which had received four or five intravenous injections of zymosan, a result which again shows that resistance to endotoxin cannot depend solely upon the phagocytic activity of the r.e.s. It is, of course, possible that defence against endotoxin involves first its clearance from the blood-stream by phagocytosis and then its retention and final destruction by the cells which have phagocytosed it. Hence, the defence would be weakened by agents which interfere with its retention or destruction as well as by agents which lessen phagocytosis. Thus it could be held that infection with B.C.G. and inoculation of zymosan prevent the retention or final destruction of endotoxin by the r.e. cells which have phagocytosed it; this they might do by so modifying the cells that endotoxin destroys them instead of being destroyed by them.

Mice at the peak of infection with E. coccoides, about five days after inoculation of a small dose of the parasite, are killed by 1/30th to 1/100th of the dose of endotoxin required to kill normal mice (Gledhill & Niven, 1957). In addition, mice infected with MHV are also much more susceptible to endotoxin than normal mice (Gledhill, 1958). Since susceptibility to endotoxin seems, in one way or another, to be influenced by agents which act upon the r.e.s., it is not unreasonable to suppose that infections with E. coccoides and MHV directly involve the r.e.s. This supposition is supported in the case of E. coccoides by the observations that infections with this parasite result in gross enlargement of the spleen and that quiescent infections are activated by splenectomy.

Mixed infections. It has been indicated that although infection of weanling mice with MHV is benign, previous or concomitant infection with E. coccoides, a harmless blood parasite, results invariably in fatal hepatitis. The enhancement of MHV infection by E. coccoides was explained as follows (Gledhill & Dick, 1955; Gledhill, 1956). MHV is considered to produce a benign disease in mice because the majority of infections of cells are latent. Cells infected in this way are not destroyed and resist further infection. E. coccoides so alters cell metabolism that in mice inoculated with this parasite the majority of cells infected with MHV are actively infected and destroyed. Since the liver parenchyma cells of mice dying of the dual infection are destroyed, the hypothesis was naturally envisaged in terms of these cells. In view of the likelihood that E. coccoides acts upon cells of the r.e.s., probably the hypothesis ought to be envisaged in terms of these cells rather than of the liver parenchyma cells. Thus, the r.e. cells of normal mice retain the virus particles, while in mice infected with E. coccoides the phagocytosed virus particles multiply and finally block the r.e.s.

Bekierkunst & Sulitzeanu (1958) have shown that the resistance of mice to infection with Brucella abortus is increased by vaccination with B.C.G. and Mika et al. (1954) have shown that the resistance of mice to the pathogenic action of Rickettsia burneti is increased by infection with

Br. suis. Pullinger (1936) found that guinea-pigs infected with tuberculosis are more resistant to brucellosis and others have found that infection of guinea-pigs and mice with Mycobacterium tuberculosis increases resistance to a variety of infections, e.g. Bacillus anthracis and Br. suis (Henderson et al., 1956); Staphylococcus aureus (Dubos & Schaedler, 1957); Pasteurella pestis (Girard & Grumbach, 1958); S. enteritidis (Howard et al., 1959). Recent experiments appear to indicate that chronic infection of mice with M. tuberculosis may raise their resistance to the lethal effect of ectromelia (Gledhill & Rees, unpublished data). The mechanism responsible for these effects is not understood. It would appear probable that the first inoculated agent in some way interferes with the intracellular multiplication of the second agent. The hypothesis that the interference is due to the capacity of the first agent to set in train the production of substances which raise cellular resistance might be worth investigation.

Summary and conclusions. The use of substances which increase the level of non-specific resistance to infectious diseases has an obvious place, especially in the diseases caused by agents of multiple antigenic types and in the many milder infectious diseases for which the classical methods of specific vaccination are not practicable. Hence, investigations to discover the mechanisms which determine non-specific resistance to infectious disease are required. Inoculations of suspensions of diverse substances whose particle size is such that they are taken up by the phagocytic cells of the reticulo-endothelial system (r.e.s.) often have a marked influence upon resistance to infectious diseases. The changes of resistance and the changes of phagocytic activity of the r.e.s. produced by such substances show a general correspondence and thus it is natural to believe that the phagocytic activity of the r.e.s. plays an important part in resistance to infectious diseases. There are exceptions to this postulated rule some of which are indicated in the Table: others include the observations that prior infection with Eperythrozoon coccoides greatly increases susceptibility to the pathogenic effect of murine hepatitis virus and treatment with E. coccoides, B.C.G. or zymosan drastically increases susceptibility of mice to bacterial endotoxin at a time when the phagocytic activity of the r.e.s. is known to be higher than normal, at least in the case of B.C.G. and zymosan. These exceptions can be met by recognizing that prevention of the pathogenic effects of noxious agents requires not only their phagocytosis but also their subsequent destruction, or at least their retention in a situation where they are harmless. Thus, inoculations of Thorotrast increase the phagocytosis of Salmonella typhi-murium but since they do not inhibit its intracellular multiplication, phagocytosis does not lower its pathogenicity. Similarly, inoculation of endotoxin leads to increased multiplication of ectromelia virus in the cells which have phagocytosed it and thereby increases its pathogenicity. In other words, it must be assumed that any material which stimulates phagocytosis by r.e. cells may also modify these cells in such a manner that their resistance is increased for some pathogens and decreased for others. Since serum from mice reacting to endotoxin, MHV and ultraviolet inactivated influenza virus and serum from mice maintained in a hot

environment all show a similar sparing effect on ectromelia it is possible that adverse conditions lead to the production of a substance which raises resistance to infection with this virus. As the substance has no direct effect upon the virus, it probably acts by raising resistance of cells to the pathogenic effects of the virus. Since tissues contain polysaccharides somewhat similar to endotoxin, it is possible that very small doses of endotoxin (unlike larger doses) directly raise the resistance of cells to the pathogenic effects of viruses and that cells exposed to adverse conditions of various kinds release endogenous polysaccharides which themselves act in the same way as small doses of endotoxin. These questions and possibilities, wherever the truth may lie, suggest the existence of a mechanism of defence which, although inadequate to check overwhelming infections such as fall within the ambit of classical immunity, can exert an important effect upon lesser infections.

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Magnesium Homeostasis and Hypomagnesaemia in Ruminants

BY

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The problem of hypomagnesaemia was last reviewed in this journal by Blaxter & McGill (1956). That review covered the literature up to 1955, and dealt with the work of Bartlett, Brown, Foot, Rowland, Allcroft & Parr (1954) on the effect of nitrogenous fertilizers, and of Head & Rook (1955) on the relation of ruminal ammonia to hypomagnesaemia in cattle. It was known, also, that in many cases the hypomagnesaemia associated with spring grazing could be prevented by feeding supplements of magnesium oxide or calcined magnesite (Allcroft, R., 1954).

Work on these lines has been continued and expanded by workers at the National Institute for Research in Dairying and The Central Veterinary Laboratory, and considerable attention has been paid to the "nutritional" aspects of hypomagnesaemia and means whereby the uptake of magnesium may be increased. Workers in Holland and Norway have reported further experimental work on hypomagnesaemia in cattle, laying emphasis on different aspects of the problem. Seekles (1958) has suggested that the condition is better described as 'Nutrition Tetany' while Kemp & T'Hart (1957) have emphasized the role of potassium or the ratio $\frac{K}{Ca + Mg}$ in diet as an aetiological factor. All workers seem to be agreed on the efficacy of magnesium supplements as a preventive measure.

During this period there have been signs of an increased interest in magnesium metabolism in the medical field; hypomagnesaemia and magnesium deficiency have been observed in chronic alcoholism and after prolonged parenteral fluid infusions. Hypermagnesaemia has been observed in certain cases of uraemia. The availability of a radioactive isotope, ^{28}Mg, has led some workers to take an interest in the subject.

This review will not deal with such questions as the incidence and clinical syndrome of hypomagnesaemia but will consider some of the fundamental aspects of magnesium metabolism and try to relate these to the problem in ruminants.

Distribution and Interchange of Magnesium between Tissues

Magnesium is distributed throughout all the tissues of the body. Like potassium it is primarily an intracellular ion, though it occurs in the extracellular fluid and the skeleton. According to various authors (see Harris, 1956; and Blaxter et al., 1954), the highest concentrations of Mg occur in heart and skeletal muscle. Liver, kidney, brain and lung have much lower concentrations expressed as mg. or meq. (milli-equivalents) per unit wet weight. If these concentrations are re-calculated on

the basis of intracellular water (where sufficient ancillary data are given), then the differences between heart, skeletal muscle, liver and brain, are much smaller. In fact, muscle, heart, liver and brain are very close, while kidney has a rather higher concentration. The average value is about 30 meq. of Mg per litre of intracellular water.

The concentration of Mg in the extracellular fluid (ECF) is much lower than inside the cell. In most animals it is about 2 meq./l. (2.5 mg./100 ml.). This is not the place for a review of the methods of determination of Mg in biological materials; suffice it to say that there is a wide variety of methods, none of which is generally accepted. This alone will account for some of the discrepancies between different reports. The generally accepted normal range for plasma Mg concentration is 1.6 to 2.5 meq./l. (2.0–3.5 mg.%). In view of its importance in relation to hypomagnesaemia, discussion of extracellular Mg will be reserved for a later section.

The state of magnesium inside the cell is a matter for some debate, as is the mechanism by which it enters or leaves cells. It was assumed that most of the Mg in the cell existed in a "bound" or "complexed" form, or that it was not easily exchangeable as a result of a permeability barrier. Harris (1956) reported that soaking tissues in solutions with a wide range of Mg concentrations had little effect on the intracellular concentration. In view of its importance as a co-factor in numerous intracellular enzyme systems, it is not surprising that the intracellular Mg should appear to be relatively unexchangeable. The present author once held the view that magnesium entered cells only when required for the synthesis or maintenance of cell enzymes.

More recently, the availability of radioactive magnesium (^{28}Mg) has permitted more direct studies of the distribution of Mg in the tissues. Brandt et al. (1958), using dogs, and MacIntyre (1959), using rats, have shown that some at least of the intracellular Mg is readily exchangeable. Hence there is no insuperable permeability barrier and there must be constant movement of magnesium into and out of cells.

The rate at which ^{28}Mg exchanges, varies in different tissues. Brandt et al. (1958) found that the uptake of ^{28}Mg was most rapid in the heart, then liver and kidney. The rate of uptake was considerably slower in skeletal muscle and bone. MacIntyre (1959) on the basis of specific activity curves found the exchange rate was most rapid in liver. He interpreted the results to show that in muscle there are two forms of magnesium, one rapidly, and one more slowly exchangeable. From the similarity of the specific activity time curves it might be presumed that brain also contains two forms of Mg.

Although studies with ^{28}Mg have demonstrated that cell magnesium crosses the cell membrane, no light has yet been thrown on the mechanism by which the large concentration gradient between intracellular and extracellular Mg is maintained. In view of MacIntyre's results it is interesting to speculate that in muscle the more rapidly exchangeable Mg is ionic Mg in the intracellular water in physicochemical equilibrium with that in the extracellular water and that the slower component represents Mg which is bound by the intracellular anions and therefore, less rapidly exchangeable. However, it is difficult

to reconcile this suggestion with Harris's report on the effect of soaking tissues in solutions containing different concentrations of magnesium, and at the present it seems most likely that the entry of magnesium into the cells is the result of an active process.

Using sheep erythrocytes in vitro, Care et al. (1959) have shown that ^{28}Mg can enter the red cell under suitable conditions, which suggests that there is a flux of Mg ions across the cell membrane. In animals, other than the ox, Mg occurs at a higher concentration in erythrocytes than in plasma. Salt (1950) suggested that the concentration of erythrocyte Mg was related to the plasma concentration at the time the erythrocytes were formed. Recent work has demonstrated some interesting relations in man. Nabarro et al. (1952) studied cases of severe diabetic ketosis in man and found evidence of cellular depletion of Mg despite normal plasma concentrations. Flink et al. (1957) suggested that in magnesium deficiency in man, intracellular depletion of Mg was the important factor in the production of symptoms. Smith et al. (1958) studied cases of delirium tremens and uraemia. In a series of normal adults, the mean plasma Mg concentration was 1.8 ± 1.3 meq./l., and in the erythrocytes 5.29 ± 0.34 meq./l. In 12 cases of delirium tremens, plasma Mg was 1.5 ± 0.34 meq./l., and erythrocyte Mg 3.19 ± 0.75 meq./l. In 14 cases of uraemia with central nervous depression, the plasma Mg was 3.17 ± 1.3 meq./l. and the erythrocyte Mg was 8.84 ± 1.7 meq./l. Though the erythrocyte Mg level was high in all the cases, in four the plasma concentration was normal. Clinical signs were more closely correlated with erythrocyte Mg than with plasma Mg concentration. These observations suggest that more attention should be paid to intracellular magnesium in animals, as they suggest a more mobile role for cell Mg than that which has been accepted. MacIntyre & Davidsson (1958) have shown, in contradistinction to the previous work of Watchorn & McCance (1937) and Blaxter et al. (1954), that the concentration of Mg in muscle falls during the development of magnesium deficiency in rats; it is interesting that the concentration of potassium in the muscle falls also, and that the ratio of K to Mg remains substantially constant.

Various workers (see Duckworth & Godden, 1940; Blaxter et al., 1954) have reported that in magnesium deficiency there is a loss of about 30% of the Mg from bone. It is suggested that this magnesium is surface limited in the bone crystal and that it forms a mobilizable reserve of magnesium. Experiments with ^{28}Mg show that Mg in bone is only slowly exchangeable in adult rats and dogs (MacIntyre, 1959; Brandt et al., 1958). While considering the distribution of Mg in tissues, it is convenient to consider another aspect of magnesium deficiency, i.e., its relation to calcium in tissues. Blaxter et al. (1954) found no evidence of calcification in the vascular system of magnesium deficient calves. MacIntyre & Davidsson (1958) and Wickham (1958) have found that in rats and guinea-pigs nephrocalcinosis occurs in magnesium deficiency. MacIntyre (1959) suggested that magnesium is important in the transport of calcium in the renal tubule and even short periods of hypomagnesaemia may be deleterious.

Magnesium in the Extracellular Fluid (ECF)

As magnesium is present in the ECF, all the cells of the body are bathed with magnesium and this extracellular Mg can affect the activities of cells and tissues externally as well as by its intracellular function. Extracellular Mg then has two functions: (1) it is in transport to the cells; (2) it acts directly on cells and tissues as part of their environment. The ECF is divided into plasma and interstitial fluid. The latter is an ultrafiltrate of the former, generally modified by Donnan factors, as the protein concentration of plasma is much greater than that of the interstitial fluid.

In the study of living animals the only easily observable magnesium is that in the plasma. Plasma Mg can be divided into ultrafiltrable and protein bound Mg. Present evidence suggests that the ultrafiltrable magnesium is all ionic, unlike plasma calcium where there is a non-ionic ultrafiltrable fraction (Copeland & Sunderman, 1952). The proportion of the magnesium which is ionic is important as it is the physiologically active form.

The relation between magnesium and protein in plasma is governed by the Law of Mass Action

$$MgProt \rightleftharpoons Mg^{++} + Prot^{=}$$

for which equilibrium the dissociation constant is

$$K = \frac{(Conc.\,Mg^{++})(Conc.\,Prot^{=})}{(Conc.\,MgProt)} \qquad \text{and } pK = \log_{10}\frac{1}{K}$$

(Copeland & Sunderman, 1952; Carr & Woods, 1955).

Carr & Woods (1955) studied the reaction between magnesium and various proteins, and found that albumin bound Mg most strongly. At pH 7.4, about 10 moles of magnesium were bound per 100,000 g. of albumin (8 moles/mole), the figure for globulin was about 6 moles per 100,000 g. Binding was influenced by pH, being maximal at pH 10 while at pH 6.0 there was little or no binding. Between pH 7.0 and 7.5 the amount bound increased by 50% This was not under conditions of maximum binding nor at physiological concentrations. From their results they calculated that pK was 2.37 ± 0.09 which is not significantly different from the pK for calcium binding 2.43 ± 0.04.

Since the dissociation constants for magnesium and calcium proteinates are so closely similar, Carr (1955) suggested that Mg and Ca compete for the binding sites on plasma proteins. If this is so, then

$$\frac{(Conc.\,Ca\ ions)}{(Conc.\,Mg\ ions)} = \frac{(Conc.\,Ca\ Proteinate)}{(Conc.\,Mg\ Proteinate)}$$

Using crystalline albumin and the technique of equilibrium dialysis, Carr showed that this relation held within the limits of experimental error. This result suggests that in solutions containing both Ca and Mg the relative binding of the two ions should be proportional to their relative concentrations. If this is true for plasma it has interesting implications in the study of bovine hypomagnesaemia and milk fever. It is dangerous to assume that results obtained in purely physico-chemical systems apply in the plasma of living animals, however, some preliminary results on the competitive binding of Mg and Ca by 'plasma', using the same technique as Carr, gave similar results to those obtained with pure

albumin (Wilson, A. A., unpublished). It has yet to be shown that these relations obtain in vivo.

It is reasonable to assume that the concentration of Mg or Ca in the interstitial fluid will be very close to that of ultrafiltrable Mg or Ca in plasma and that this will be the physiologically active concentration. While it is generally accepted that about 50% of the plasma Ca is ionic, there is no such general agreement regarding plasma Mg, and values between 20% and 50% have been given as the fraction of bound Mg in plasma. The most recent mean figure for human plasma is that 35% of the magnesium is bound to protein (Prasad et al., 1958).

Though homeostasis of magnesium will be considered later, this is a suitable place to mention some of the factors which have been reported to have an influence on the plasma Mg concentration. In contradistinction to calcium, no one endocrine gland has been shown to exert a primary regulating effect over plasma magnesium concentration. Nevertheless some workers have shown that there are interrelations between plasma Mg concentration and the thyroid and adrenal cortex.

Weil & State (1958) examined the effect of removing various endocrine glands from dogs. After adrenalectomy the plasma Mg concentration fell for two days then rose steadily until death. This confirmed the earlier results of Harrop et al. (1933) and of Zwemer & Sullivan (1934). When the dogs were subjected to thyro-parathyroidectomy the plasma Mg concentration fell, remained low for about 20 days and then returned to normal. At the same time the calcium concentration fell and tetany was observed after the fifth day, but this sign disappeared when the Mg concentration returned to normal. Other work on the effect of thyroidectomy on the plasma Mg concentration has led to equivocal results. Soffer et al. (1941) found that in thyroidectomized dogs and myxoedematous human beings, the ultrafiltrable fraction of plasma Mg was increased, in some cases to 100%. Dine & Lavietes (1942) confirmed this abnormal ratio of bound and free magnesium but other workers failed to do so (see Silverman & Gardner, 1954).

Low concentrations of plasma Mg were reported in a human case of primary aldosteronism due to an adrenal tumour (Mader & Iseri, 1955). Flink et al. (1957) have suggested that the low plasma Mg concentration seen in cases of hepatic cirrhosis is the result of secondary hyperaldosteronism. Hyperthyroidism was reported by Soffer et al. (1939) to be accompanied by a decreased proportion of ultrafiltrable plasma Mg though the total concentration was normal. This was not confirmed by Silverman & Gardner (1954). Swan & Jamieson (1956b) found that feeding thyroprotein to milking cows led to a fall in the plasma Mg concentration; they made no observations on the proportions of bound and free Mg. Further work on this relation between the thyroid and magnesium binding is necessary.

Vitale and his co-workers have studied the relation between thyroxine and Mg in growing rats (Vitale et al., 1957; Gershoff et al., 1958). They found that the depression of growth rate and the hypomagnesaemia which result from thyroxine feeding, can be partly (80%) reversed by feeding additional Mg. Since the growth rate is depressed, it is unlikely that the hypomagnesaemia is the result of depletion of the Mg

stores in order to supply the needs of growing soft tissues.

Absorption and Utilization of Dietary Magnesium

Despite the probability that the rate of absorption from the intestine plays an important part in the regulation of Mg metabolism, there have been very few reports of direct investigation into the mechanisms involved. Most of the information available is indirect and arises from studies concerned with the availability and utilization of dietary Mg by animals.

Stewart & Moodie (1956) studied the absorption of Mg by anaesthetized sheep. They compared the Mg concentration of arterial blood with that of blood from the veins draining different parts of the gastro-intestinal tract. Large doses of Mg salts were given orally or by infusion into specific parts of the tract. They found that, under these circumstances, absorption of Mg appeared to occur from the rumen, abomasum, small intestine and caecum. The principal sites of absorption appeared to be the duodenum and small intestine. They found also that magnesium nitrate was more rapidly and efficiently absorbed than magnesium sulphate. (Work in this laboratory using in vitro techniques for studying the absorption by rat small intestine failed to show any difference between the nitrate, sulphate, chloride or acetate of magnesium (Ross, 1959).

Smith (1959) found that up to five weeks of age Mg was absorbed from the large intestine of calves, but after this age there was no further absorption and the efficiency of utilization fell considerably. McCance & Widdowson (1942) claimed that the urinary excretion of Mg in man reflected the intestinal absorption. This finding has been used to make deductions regarding absorption from observations of the urinary excretion of Mg. Head & Rook (1955) suggested that the fall in urinary Mg which follows the feeding of spring herbage to cows is the result of decreased absorption of dietary Mg. Aikawa et al. (1958) and Aikawa (1959) fed ^{28}Mg to man and rabbits, respectively, and suggested that the low level of renal excretion was the result of poor gastro-intestinal absorption.

Rook et al. (1958) and Rook & Balch (1958) have studied the availability of Mg to cows on various diets. Dietary Mg which is not excreted in the faeces is termed the 'available' Mg and the proportion of dietary Mg which is available is termed the 'availability'. They found that the 'availability' of Mg showed wide variation when different, typical winter rations were fed to milking cows. The 'availability' was greatest on a ration of hay plus dairy cubes and lowest on a ration of hay, dairy cubes and decorticated ground-nut meal. On rations consisting of hay, concentrates and silage or mangolds, the 'availability' was intermediate. When the cows were transferred abruptly to spring herbage, both the intake and the 'availability' of the Mg were lessened. The change was followed by an immediate fall in the urinary Mg output and a progressive development of hypomagnesaemia. The factors responsible for the difference in the 'availability' of Mg on different rations, particularly the difference between stall rations and herbage, have yet to be determined. Rook & Balch (1958) drew attention to some of the suggestions made, viz. high

potash content of the herbage (Sjollema, 1932), abnormal anion–cation balance (Brouwer, 1952) or high nitrogen content (Head & Rook, 1957). They pointed out that these factors may act alone or in concert.

Studies of the absorption of Mg are complicated by the fact that the Mg in the gastro-intestinal tract is not solely dietary in origin. All animals produce large volumes of digestive secretions containing a considerable amount of endogenous Mg. Hence the 'available' Mg as defined by Rook et al. is really the Mg absorbed less the endogenous Mg, and a change in 'availability' may be due either to decreased absorption or to increased production of endogenous Mg.

Field et al. (1958) studied magnesium balance in two wethers. Their technique enabled them to estimate the daily endogenous Mg and to allow for this when calculating the efficiency of absorption. They found a marked difference between the two animals in the endogenous Mg and in the efficiency of absorption. On the other hand they found no difference in the efficiency of absorption of Mg from herbage samples, whether cut from fields on which cows had developed grass tetany, normal fields, autumn pasture or grass nuts. Field (1959) used ^{28}Mg to determine the endogenous Mg and efficiency of absorption in a wether, and found values very similar to those of Field et al. (1958). Macdonald et al. (1959) also used radioactive Mg and by different technique found essentially the same values as Field for endogenous Mg and efficiency of absorption.

If the rate of absorption of Mg has a role in magnesium homeostasis then the amount of endogenous Mg entering the gastro-intestinal tract is of considerable importance. On theoretical grounds the present author (Wilson, 1958) suggested that a decrease in the absorption rate could lead to a considerable loss of extracellular Mg in 24 hours. On the basis of the results of Field (1959), Field et al. (1958) and Macdonald et al. (1959) the amount of endogenous Mg entering the gastro-intestinal tract in 24 hours was not less than the total extracellular Mg. The total extracellular Mg was calculated from the plasma concentration and the assumption that the ECF volume is 20% of the body weight. The factors which influence the production of endogenous Mg have not been studied but two possible factors are, the total volume of the digestive secretions produced and the plasma or rather extracellular, concentration of Mg.

The importance of absorption in the regulation of extracellular Mg is implicit in the observation that hypomagnesaemic tetany may be prevented by feeding supplements of magnesium oxide or calcined magnesite (Allcroft, R., 1954; Line et al., 1958) or increasing the Mg content of pasture by treatment with magnesite or magnesian limestone (Parr & Allcroft, 1957). The importance of absorption is also implicit in the observation of Inglis et al. (1959) that fasting sheep develop hypomagnesaemia.

The Excretion of Magnesium

Magnesium absorbed in excess of the body's requirements is excreted by the kidney, though there is some doubt as to the mechanisms involved. A considerable amount of endogenous Mg may be lost in the faeces; this has already been considered.

The observed facts of the urinary output of Mg can be described in terms of a filtration-reabsorption mechanism, thus

$$U_{Mg}.V = P_{Mg}.F - T_{Mg}$$

U_{Mg} is the concentration of Mg in the urine, V is the volume of urine produced per min., F is the glomerular filtration rate. P_{Mg} is the concentration of Mg in the plasma and T_{Mg} is the maximum tubular reabsorption rate in mg. per min., if F is in ml. per min. and P_{Mg} is in mg. per ml. Magnesium, then, is a threshold substance and only appears in the urine if the load, $P_{Mg}.F$ is greater than the maximum tubular reabsorption rate, T_{Mg}. The maximum tubular reabsorption rate is usually taken to be a physiological 'constant' (though this has yet to be proved for Mg) and in conjunction with the filtration rate fixes the threshold concentration in the plasma. Preliminary experiments in the author's laboratory suggest that in ewes the value of T_{Mg} is about 2 mg./min.; as the filtration rate in these particular ewes is about 100 ml./min., the threshold concentration in plasma is about 2 mg./100 ml. As could be expected, Rook et al. (1958) found a correlation between the serum concentration and urinary output of Mg; they calculated a regression equation relating the serum Mg concentration to the daily urinary output. (A renal physiologist would prefer to show the urinary output as a function of the serum concentration as the plasma is the source of all the urinary Mg and not vice versa.) From their regression equation Rook et al. estimate the renal threshold for plasma Mg in cows to be not greater than 2.15 mg./100 ml.

On the basis of an average plasma Mg concentration of 2.5 mg./100 ml., the amount of Mg filtered and reabsorbed in 24 hours is more than ten times the total amount of Mg in the ECF. In view of this it is obvious that quite small changes in either filtration or reabsorption rates could be the cause of considerable changes in Mg balance. On the basis of Poulsen's (1957) study of the filtration rate in cows, and with a renal threshold of 2 mg./100 ml., it can be shown that a fall in the filtration rate or an increase in the tubular reabsorption rate of 10%, will lead to a fall in the daily urinary output of Mg from 4 to 1.75 g./day. Since little is known of the factors influencing renal dynamics in ruminants, these considerations of filtration and reabsorption should be borne in mind when interpreting changes in urinary output as indicative of changes in intestinal absorption, particularly in the case of animals at pasture.

So far it has been assumed that a filtration-reabsorption mechanism is adequate to describe the renal excretion of Mg. However, it has been shown that the aglomerular teleost fishes actively excrete Mg through their tubules (Bieter, 1933; Berglund & Forster, 1958). This observation has led to suggestions that there may be active tubular excretion of Mg in other animals. The evidence for tubular excretion of Mg in mammals is somewhat conflicting. Heller et al. (1953), Jabir et al. (1957) and Barker et al. (1959) all found that intravenous infusion of magnesium salts in man decreased the urinary excretion rate of potassium. As there is evidence for the active tubular excretion of potassium (Berliner et al. 1950), the observed decrease in the urinary excretion rate of potassium may be the result of competition between Mg and K for a transport mechanism. Chesley & Tepper (1958) found, however, that the effect of Mg on

potassium excretion varied in different subjects and Womersley (1958) found no change in daily output of potassium as a result of two infusions of magnesium lactate per day. The present author has found that following a single intravenous injection of a magnesium salt there is a decreased output of urinary potassium in ewes.

Barker et al. (1957) used dogs to study the effect of large intravenous loads of Mg. With high levels of loading the Mg excreted exceeded that filtered by about 10% and this is suggestive of tubular excretion. However these experiments were limited by the fact that at the high levels of plasma Mg concentration necessary to show this tubular excretion, the condition of the dogs deteriorated rapidly. A number of workers have used the 'stop-flow' technique of Malvin et al. (1958). Ginn et al. (1959) found that the excretion pattern of ^{28}Mg was suggestive of the secretion of Mg by the distal tubule. Murdaugh & Robinson (1959) in similar experiments obtained results which suggested that Mg was added to the glomerular filtrate in the distal tubule, but pointed out that this may have been due to diffusion and not necessarily to active secretion. Wesson & Lauler (1959) in their 'stop-flow' experiments found no evidence for the tubular excretion of Mg or calcium. The question thus remains an open one, and for the time being it is simpler to assume that the renal handling of Mg is by a filtration-reabsorption mechanism. Some of the workers already cited have quoted results which suggest that there may be interrelations between the excretion of Mg, Ca, Na etc., but no clear picture emerges and it is better to conclude with Barker et al. (1959) that excretion of Mg is by a filtration-reabsorption mechanism in which the tubular reabsorption process is acting at or near its maximum rate and that the excretion of Mg is partly or wholly independent of the excretion of other ions.

DISCUSSION

Although there has been considerable speculation and experimental work on the cause of clinical hypomagnesaemia, much less effort has been devoted to the fundamental problem, namely the regulation of the concentration of magnesium in the extracellular fluid. The maintenance of a normal concentration of Mg in plasma is essentially a matter of regulating the amount of magnesium in the ECF. Though the concentration of Mg in plasma is greater than that in the interstitial compartment of the ECF, the concentration of the physiologically active, ionic Mg is probably uniform throughout both compartments; the higher concentration in plasma being the result of binding by the proteins present. Homeostatic mechanisms are probably directed to maintaining the constancy of the ionic Mg in the ECF as a whole; the binding of some of the Mg by plasma protein simply increases the total amount required.

The available evidence does not suggest that the homeostasis of Mg is under the control of any one particular endocrine gland, though there is evidence of some interrelation with the thyroids and the adrenal cortex. As far as ruminants are concerned, there is considerable evidence to suggest that magnesium from the gastro-intestinal tract plays an important part in keeping up the concentration of Mg in the ECF. Less

attention has been paid to the mechanisms which prevent the Mg concentration from rising too high, though from the physiological point of view, this is very important. Such information as is available on this point comes mainly from studies of the results of intravenous infusions of various salts of Mg in dogs and man. As the Mg in these cases appears to be rapidly excreted in the urine, it seems likely that the kidney is primarily responsible for preventing the Mg concentration in the plasma rising too high. Hence in the normal animal, on an adequate diet, the concentration of Mg in the ECF is probably the result of a balance, between intake from the digestive tract, and renal excretion.

It has been suggested, largely on the basis of the results of experimental magnesium deficiency, that, in the young animal at least, part of the Mg in bone forms a reserve which can be used for homeostasis when necessary (Blaxter & Rook, 1954; Smith, 1959). Although this mechanism may operate in young animals, it is less certain that it is effective in adult animals. In adult rats and dogs, MacIntyre (1959) and Brandt et al. (1958) respectively, using ^{28}Mg found that the bone Mg was only slowly exchangeable. There is no evidence that the Mg in the soft tissues can be used for homeostasis in the ECF. although experiments with ^{28}Mg have shown that part of the soft tissue Mg is exchangeable. To a first approximation then, homeostasis can be considered to be the result of a balance between intake from the intestine and renal excretion.

Robinson et al. (1958) have shown that uraemic patients develop hypermagnesaemia when the glomerular filtration rate falls below 30 ml./min.; this confirms the importance of the urinary excretion rate as a mechanism preventing too great a rise in extracellular Mg. The observation that hypomagnesaemia on spring pasture can be prevented by feeding supplements of Mg despite an adequate intake from herbage, suggests that the transfer of Mg from the intestine is an important means of preventing a fall in the plasma Mg concentration. This is confirmed by the results of Swan & Jamieson (1956b), who found that underfeeding led to a fall in the serum Mg concentration and that this fall was greatest in recently calved cows or those which maintained a high milk yield. despite the underfeeding. Inglis et al. (1959) also found that starvation in sheep led to a period of hypomagnesaemia.

The question now arises, whether in grass tetany the hypomagnesaemia is due to the Mg in herbage being less 'available', i.e., nutritional deficiency, or whether there is a change in the process of absorption. The latter would affect also the reabsorption of endogenous Mg and lead to a direct loss of extracellular Mg. Field et al. (1958) found no difference in the availability of Mg when 'grass tetany herbage' was compared with normal herbage. This suggests that 'animal factors' are important in determining the rate of Mg transfer from the intestine to the ECF. The most probable 'animal factor' is a variation in the rate of absorption, the parameters of which have yet to be determined.

This concept of extracellular Mg homeostasis as a 'steady state' which is the result of a constant flow, provides a reasonable explanation of the rapid fall in plasma Mg concentration which occurs when cows are turned out on to spring pasture. However, such a concept is probably an oversimplification and is hardly adequate to explain the acute attacks of

tetany which occur in seasonal hypomagnesaemia nor does it take into account the possible effects of endocrine organs or stress factors.

The role of the endocrine system in magnesium homeostasis has yet to be determined. Mention has already been made of the effect of the thyroid and the adrenal cortex. Thus adrenalectomy leads to hypermagnesaemia while in hyperaldosteronism, hypomagnesaemia occurs (Weil & State, 1958; Mader & Iseri, 1955). These effects of the adrenal cortex suggest a possible connexion between stress and hypomagnesaemia. The mechanisms by which the adrenal cortex influences the plasma Mg are not known, but Mader & Iseri (1955) found in their patient that there was an increase in the excretion of both urinary and faecal Mg. This is suggestive of interference with absorption mechanisms both in the intestine and the renal tubule.

Swan & Jamieson (1956b) found that feeding thyroprotein to cows led to a fall in serum Mg. As exposure to cold leads to an increase in thyroid activity, there is a possibility that the acute attacks of tetany which occur during seasonal hypomagnesaemia as a result of sudden cold weather, could be mediated by the thyroid gland (Allcroft, W. M., 1947).

The finding of Carr (1955) that calcium and magnesium compete for binding sites on protein may have some relevance. Acute attacks of tetany sometimes occur after calving during seasonal hypomagnesaemia (Allcroft, W.M., 1947). A possible explanation is that, despite the seasonal hypomagnesaemia the concentration of Mg ions is high enough to prevent tetany, but that the normal fall in plasma Ca concentration which occurs at calving frees binding sites on the proteins and reduces the concentration of Mg ions. Competition for binding sites on plasma protein would also explain some of the results of Swan & Jamieson (1956a) in which animals with a low serum Ca concentration and a normal or high serum Mg concentration were not comatose. In these cases the low serum Ca allows more of the Mg to be bound. The finding of Parr (1957) that magnesium deficient calves developed tetany only when both the serum Mg and serum Ca concentrations were low, could also be explained in terms of competition for binding sites on protein. There is no evidence that the proportions of bound and free Mg are changed in hypomagnesaemia; however, the matter is worthy of further consideration particularly in animals suffering from seasonal hypomagnesaemia, special attention being paid to auxiliary factors such as pH. The finding of Soffer et al. (1939) that hyperthyroidism increases the bound plasma Mg may be relevant.

That a simple steady state is insufficient to explain magnesium homeostasis completely is now evident. A single injection of a suitable magnesium salt is frequently sufficient to 'cure' a case of grass tetany but similar treatment has little effect on seasonal hypomagnesaemia in adult cattle or the tetany of hypomagnesaemic calves. These findings can be explained if it is assumed that there is a 'pool' of reserve Mg which exchanges only relatively slowly with that in the ECF. In seasonal hypomagnesaemia the fall in Mg concentration is slow and occurs only when the reserve pool is reduced. If, as seems possible, this reserve pool is in the bone salt (Blaxter, 1956) then it might be that the long-term homeostasis of extracellular Mg is determined by a physico-chemical

equilibrium with bone salt Mg such as has been suggested for bone salt Ca and 70% of the serum Ca concentration. On this hypothesis, when cows are turned out on to spring pasture, the loss of Mg from the ECF is so rapid that the reserve pool of Mg is unable to buffer the fall owing to the slow rate of exchange between bone salt and extracellular Mg. In seasonal hypomagnesaemia as a result of the lowered intake the reserve Mg is slowly depleted and this fixes the extracellular Mg at lower concentration. The acute exacerbations of the hypomagnesaemia which occur can be explained as a result of the stress factors already considered.

CONCLUSIONS

In this review no attempt has been made to cover all the recent work on hypomagnesaemia; rather, a selection has been made of those items which seem relevant to the general problem of magnesium homeostasis. Nevertheless the following conclusions seem to be justified.

1. The short-term homeostasis of extracellular Mg is the result of a balance, between the absorption of Mg from the intestine, which tends to raise the concentration, and renal excretion, which prevents the concentration from rising too high.
2. There is a reserve of Mg, probably in bone, but this is not readily available.
3. Endocrine and stress effects may be of importance in helping to determine 'pathological' falls in the extracellular Mg concentration.
4. As far as the hypomagnesaemia problem in ruminants is concerned, factors influencing the absorption of Mg from the gastro-intestinal tract are of great importance and call for investigation but in the overall picture of magnesium homeostasis renal, endocrine and stress effects must not be ignored.

These conclusions are based on published evidence but it is of interest that a mathematical analysis of the subject based on consideration of the rate of movement of Mg into and out of the extracellular fluid leads to the same conclusions (Wilson, A. A., unpublished).

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